MORPHOLOGY AND PIGMENTATION OF CERTAIN YEASTS FROM BRINES AND THE CUCUMBER PLANT

JOHN L. ETCHELLS, THOMAS A. BELL AND IVAN D. JONES

Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, and Departments of Animal Industry and Horticulture, North Carolina Agricultural Experiment Station, Raleigh, N. C.

CONTENTS

GENERAL INTRODUCTION [1, 2]

The purpose of this publication is to acquaint teachers, students, and research workers interested in the study of yeasts with some of our observations on the colonial and cellular morphology of the common yeast species associated with fermentations in brine.

The illustrative material shown, and hitherto unpublished, was compiled during the past several years in connection with our taxonomic studies on the principal species of yeasts associated with the gaseous fermentation of commercially brined cucumbers (*6*, *11*), as well as investigations on the identity of the types responsible for film formation on brines (*7*).

In addition to the above sources, material relating to recent work (unpublished) on the pigmented yeasts that occur on the cucumber plant has been included. Further, certain yeast species associated with meat brines are illustrated. The latter work represents a phase of investigations on meat microbiology being conducted in the Department of Animal Industry of the North Carolina Agricultural Experiment Station in cooperation with the Bureau of Animal Industry (USDA) Beltsville, Maryland.

The material presented is divided into three major parts: film-forming brine yeasts; subsurface brine yeasts; and, yeasts from the cucumber plant. Each is organized to permit ready comparison of the striking influence of cultural media on: colonial morphology; cellular morphology; film formation (for some species), and, in some instances, colonial pigmentation. In all, species and varieties of yeasts belonging to 12 genera are shown. It is our hope that the illustrative material will benefit other workers and serve to supplement the monographs, bulletins and articles on methods and classification we have found useful in our yeast work (*1*, *3*, *4*, *14*, *16*, *17*, *20*, *21*).

MEDIA AND METHODS

Because most of the cultural media used and techniques employed have been described in detail elsewhere, they will only be mentioned briefly here.

VEGETABLE-JUICE AGAR; as described by Wickerham *et al.* (*22*) and modified by Etchells and Bell (*6*). SYNTHETIC VEGETABLE-JUICE AGAR; a chemically defined medium designed to simulate vegetable-juice prepared for us by Dr. W. J. Peterson, Head, Department of Chemistry, North Carolina State College. GLUCOSE AGAR; as prepared by Etchells and Bell (*6*). GLUCOSE-SALT AGAR; as above but containing 8 percent salt by weight. SYNTHETIC AGAR-A; the glucose-mineral-salts medium used by Stelling-Dekker (*20*) plus 0.01 percent yeast extract. SYNTHETIC AGAR-B; prepared from Wickerham's (*21*) yeast nitrogen base medium as follows; heat sterilize in separate containers an equal amount of 3 percent agar, and an equal amount of double strength nitrogen base medium plus 4 percent glucose; then mix the contents of the two containers together before the agar cools and pour plates. SYNTHETIC BROTH-B; a heat sterilized, single strength, liquid form of the above medium (omit agar). Used for growing yeast cells in the tests for the presence and nature of carotenoid pigments. CORNMEAL AGAR; prepared according to Skinner *et al.* (*19*) and employed in the test for mycelium production by use of point inoculations as described by Wickerham and Rettger (*23*), and Wickerham (*21*). Salt-tolerance tests were made in the divided culture dishes of Etchells and Bell (*7*), using a liquid medium consisting of cucumber brine adjusted to cover a range from 5 to 20 percent salt by weight and fortified by the addition of glucose and ethyl alcohol in 1.0 percent amounts (*7*). Tests for growth in ethyl alcohol were made in regular culture dishes containing nutrient broth plus 3 percent ethyl alcohol as the carbon source. Stained cell preparations were made by the Kopeloff and Cohen modification of the Gram stain (*15*). Wet mount cell preparations were used to show living cells and spores from vegetable-juice agar cultures. Cells were suspended in erythrosin (1–10,000) buffered at pH 4.6, placed on a slide and the cover slip pressed down tightly and sealed with immersion oil.

[1] **The authors gratefully acknowledge the grant from the National Pickle Packers Association, Chicago, Illinois to the North Carolina Agricultural Experiment Station, that made this publication possible by underwriting the cost of reproducing the natural color and black and white photographs.**

[2] Paper No. 449 of the Journal Series of the North Carolina Agricultural Experiment Station.

FILM-FORMING BRINE YEASTS [3]

It has been mentioned in an earlier report (6) that yeasts associated with cucumber brines are divided into two general groups. Those that produce a gaseous fermentation in the brine and those that produce luxuriant, wrinkled films on the surface of brines exposed to air but sheltered from direct sunlight. It is not uncommon to find that the two groups are confused in the literature on cucumber pickling.

Film formation on 40 commercial cucumber brines obtained during 1947 and 1948 in five states (North Carolina, Georgia, Michigan, Indiana and Wisconsin) has been attributed to species of *Debaryomyces*, *Zygosaccharomyces*, *Endomycopsis*, and *Candida* (7). The predominating species found were *D. membranaefaciens* var. *Hollandicus*, and *Z. halomembranis*. They were also the most salt-tolerant. Yeasts belonging to the genus *Debaryomyces* were the most widespread and were found on brines in all five states.

A similar study was done in 1950 on the film yeasts from 23 commercial brines in Indiana, Michigan and Wisconsin (*10*). Emphasis here was placed on brines less than two months old and with salt concentrations of about 10 percent. The two predominating yeasts found were the same as obtained in the earlier study. However, the presence of cultures of *Pichia alcoholophila* and *Hansenula anomala* appeared to be related to the lower salt-content of the brines.

Salt-tolerance tests have since shown that the above two yeasts grew poorly if at all above 10 percent. The same is true for *Candida krusei* obtained from low salt-content dill pickle brines in the 1947–48 study.

In addition to brined cucumbers, film-forming yeasts are found in connection with a number of other similarly preserved foods. For example, Mrak and Bonar (*18*) investigated 28 cultures isolated from surface films on 27 samples of various brined foods (dill pickles, salt-stock pickles, Zucca melon, green olives, Sicilian olives, dill weed, cauliflower, and ham brine). They found film yeasts that belonged to three genera: *Debaryomyces*, 16 cultures; *Pichia*, 9; and *Mycoderma*, 3. The *Debaryomyces* species were the most widely distributed in the brines. They were also found to be the most salt-tolerant (up to 24%).

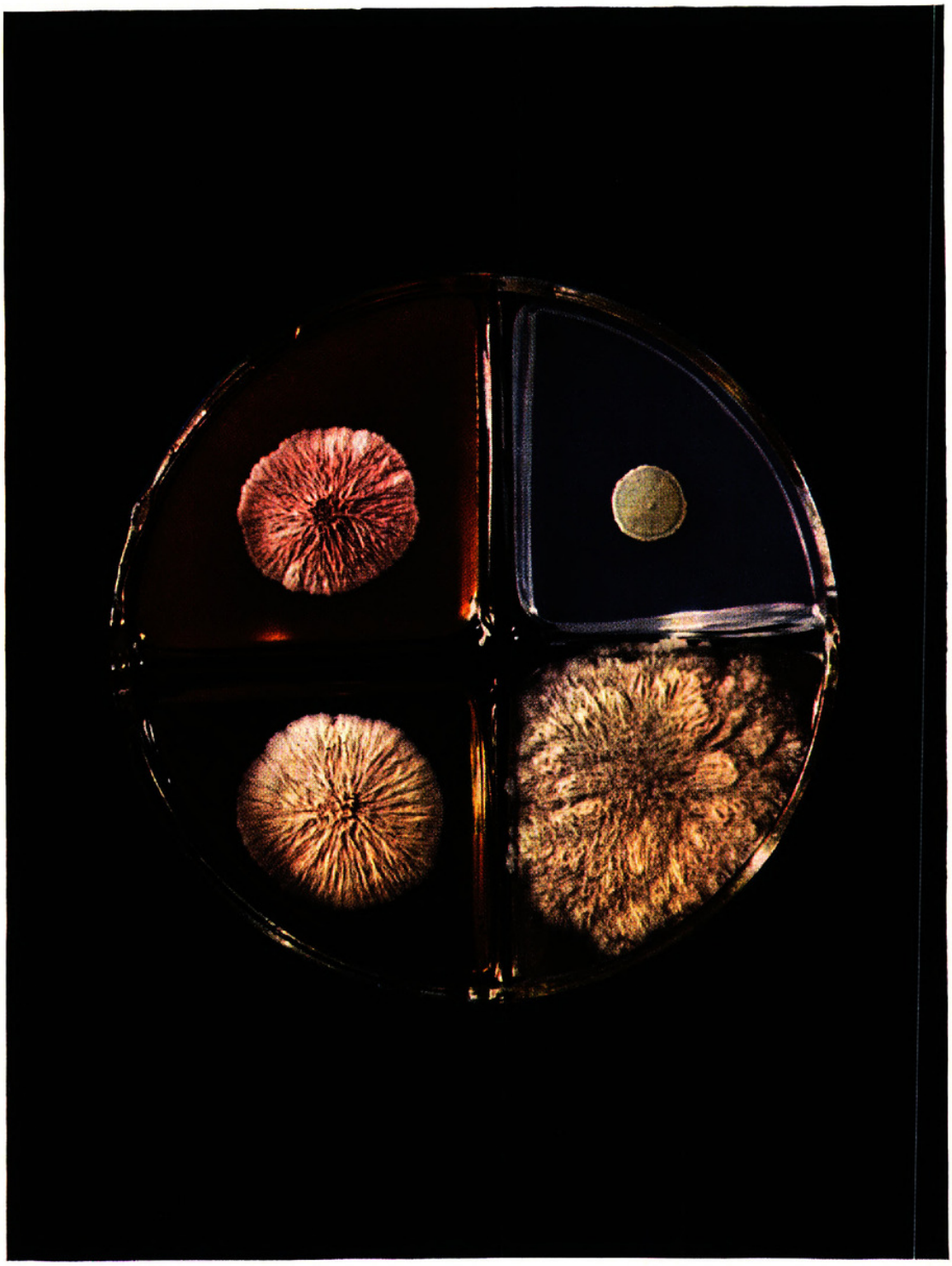
Etchells and Costilow (*9*) investigated the nature of film-forming yeasts on commercial meat brines (bacon sides, hams, beef tongues and Canadian bacon). A total of 89 yeast isolates was obtained and all were identified as belonging to the genus *Debaryomyces*. Eighty-six cultures were placed as *D. membranaefaciens* var. *Hollandicus*. The remaining three cultures were non-film-forming species that came from subsurface brine samples; these were classified as being closely related to *D. klockeri*. This yeast was also found to be the predominating type found in subsurface samples from bacon brines during a prolonged curing period.

More recently, Zenitani (*24*), isolated 29 yeast cultures from a Japanese fishery-fermentation product known as "Shiokara." Generic placement of the cultures was a follows: *Debaryomyces*, 19; *Zygosaccharomyces*, 8; *Hansenula* and *Torulaspora*, 1 each.

It is apparent that film-forming species of *Debaryomyces* are the most widely distributed yeasts associated with food brines. Other species in the approximate order of their importance would be; *Zygosaccharomyces halomembranis*, *Endomycopsis ohmeri* (and variety *minor*), *Candida krusei*, *Hansenula anomala* and *Pichia alcoholophila*.

[3] Since this article was prepared, the important new book, "The Yeasts — A Taxonomic Study," by the Dutch workers, J. Lodder and N. J. W. Kreger-Van Rij, has appeared. Thus, we have not had an opportunity to consider their proposed changes in yeast classification.

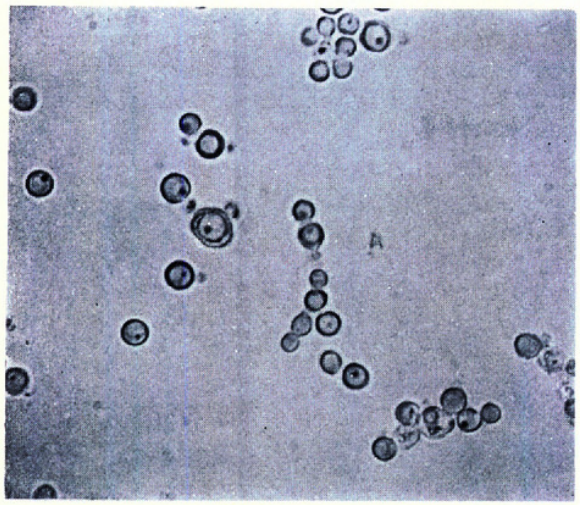
Debaryomyces



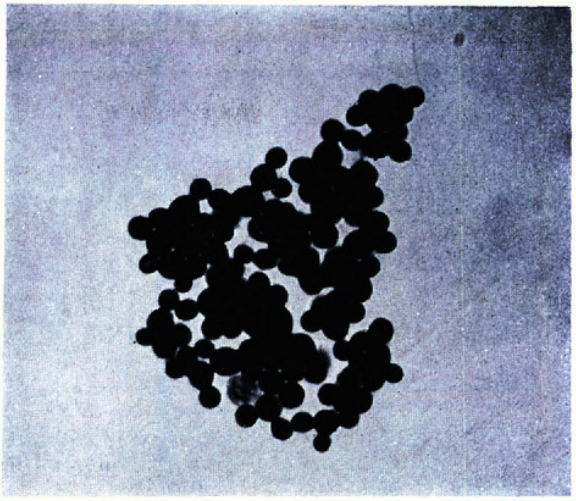
Vegetable-juice agar
Glucose agar

Synthetic agar-A
Glucose-salt agar

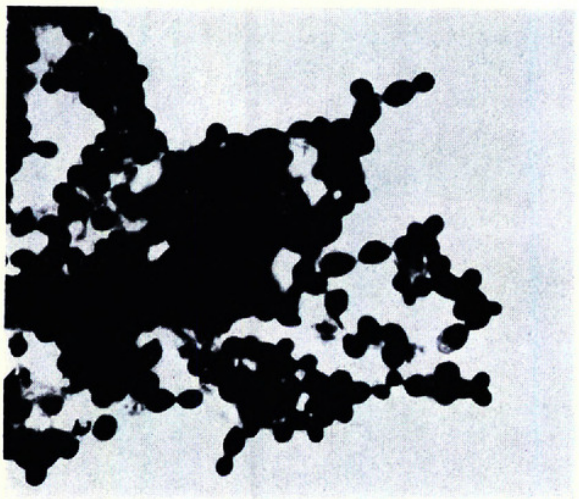
A. Comparative growth by *Debaryomyces membranaefaciens* var. *Hollandicus* Lodder (FY-36, Georgia strain) on different cultural media after 6 weeks' incubation at room temperature. Actual size. SEE OPPOSITE PAGE FOR CELLS AND GROWTH TESTS OF THIS YEAST.



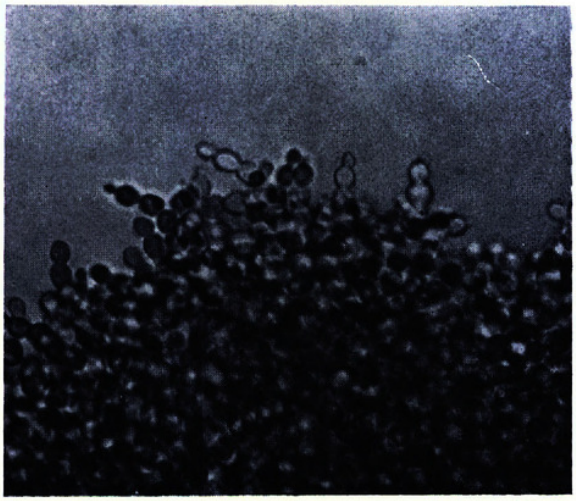
1. Sporulated cells from vegetable-juice agar at 2 months. Note rough spore (upper left); others completely fill asci. Unstained, × 1500.



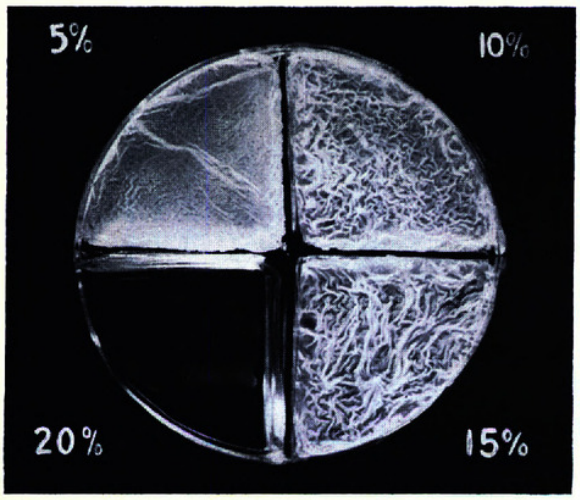
2. Round to oval cells from synthetic agar-A, 7-day old culture. Gram stained, × 1500.



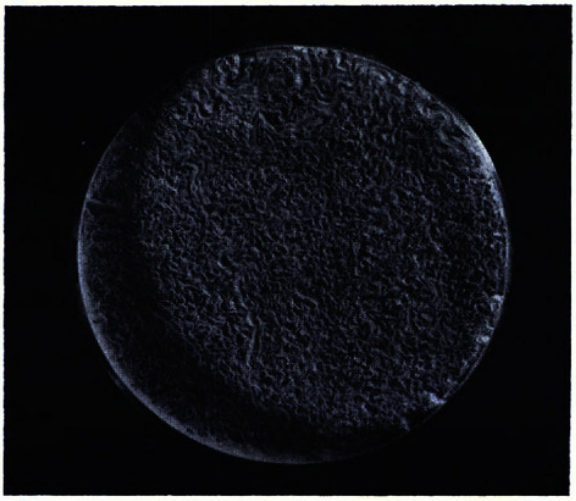
3. Cells from film on 10% salt cucumber brine, 5 days old. Gram stained, × 1500.



4. These cells show negative mycelium test on cornmeal agar at 3 weeks. Unstained, × 650; enlarged, × 2.

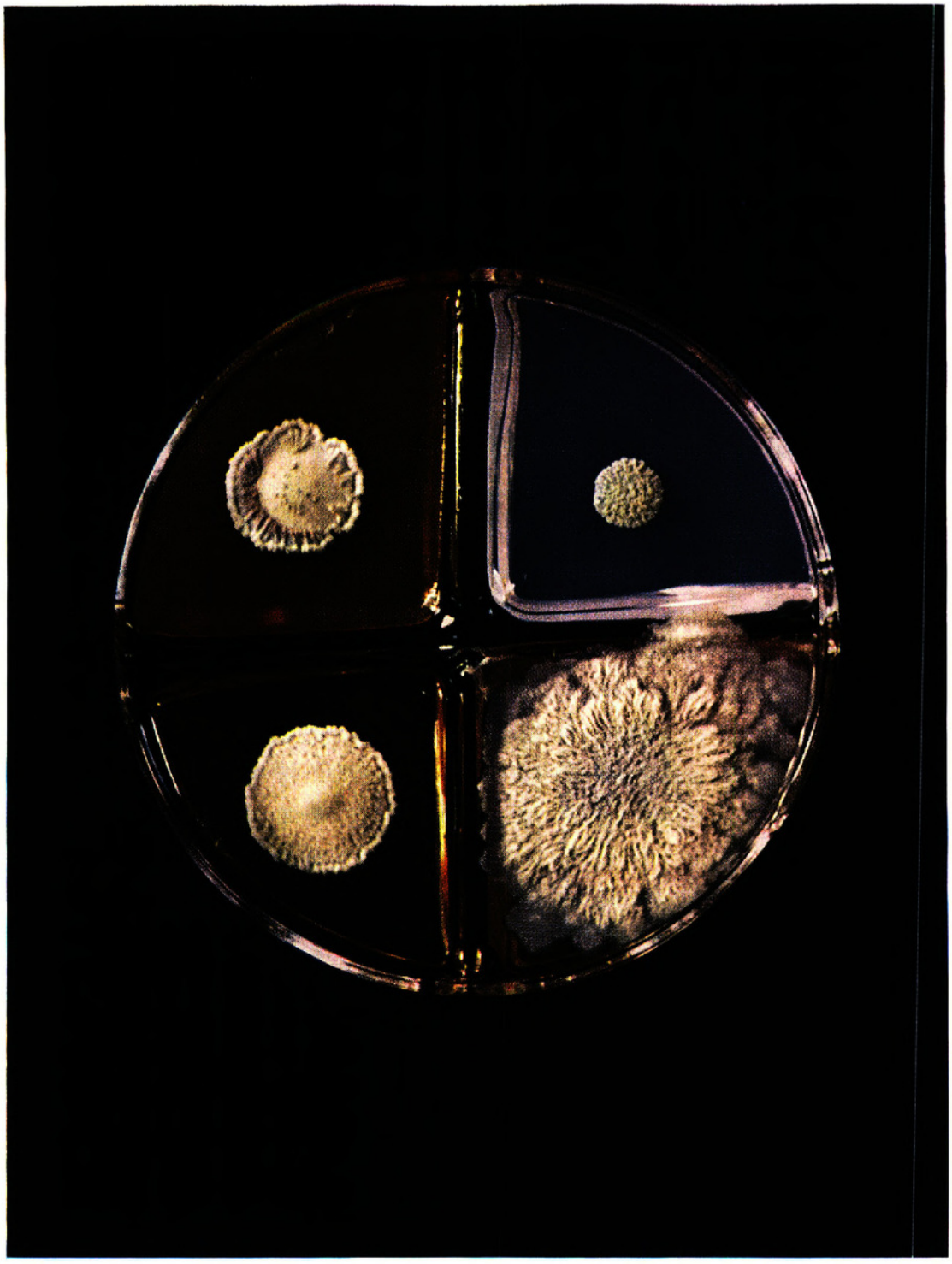


5. Salt-tolerance test at 7 days shows good growth at 3 brine concentrations; 10 days required for heavy growth at 20%. × ½.



6. Heavy film formation on ethyl alcohol medium at 4 days. × ½.

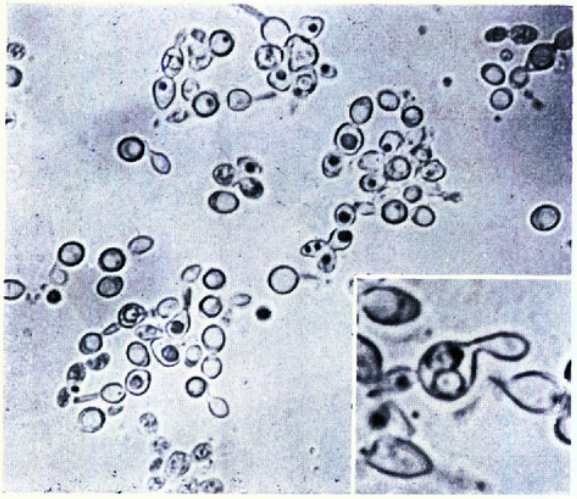
Debaryomyces



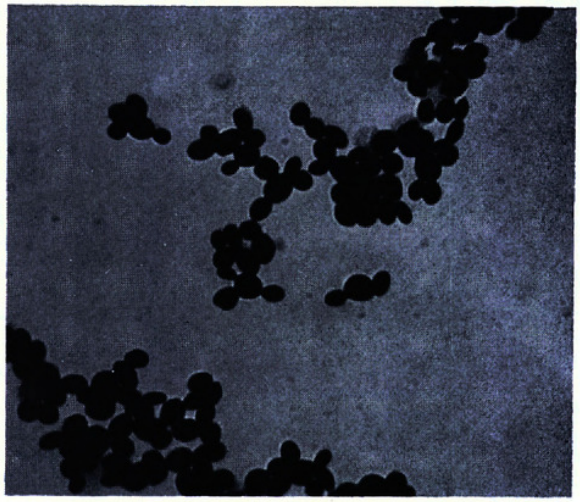
Vegetable-juice agar
Glucose agar

Synthetic agar-A
Glucose-salt agar

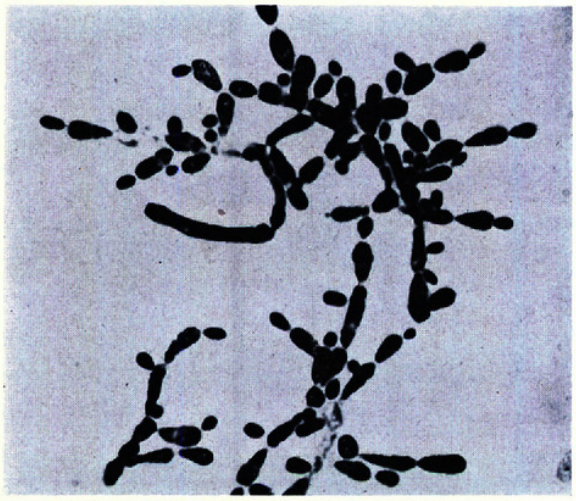
B. Comparative growth by *Debaryomyces membranaefaciens* var. *Hollandicus* Lodder (NFY-20, Wisconsin strain) on different cultural media after 6 weeks' incubation at room temperature. Actual size. SEE OPPOSITE PAGE FOR CELLS AND GROWTH TESTS OF THIS YEAST.



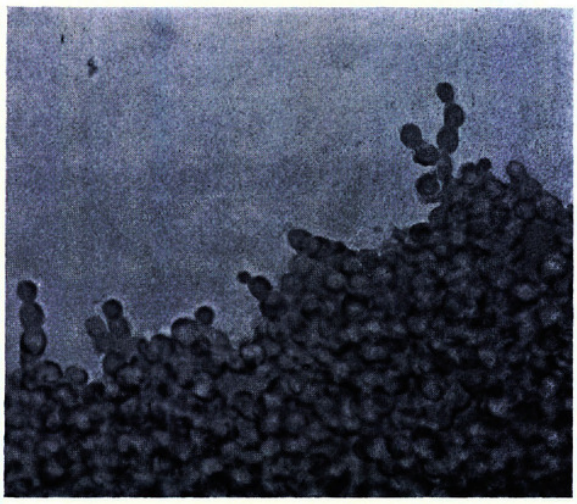
7. Tubular cells and single spores from vegetable-juice agar at 2 months. Unstained, × 1500. Insert; ascus with 2 spores, enlarged, × 2.



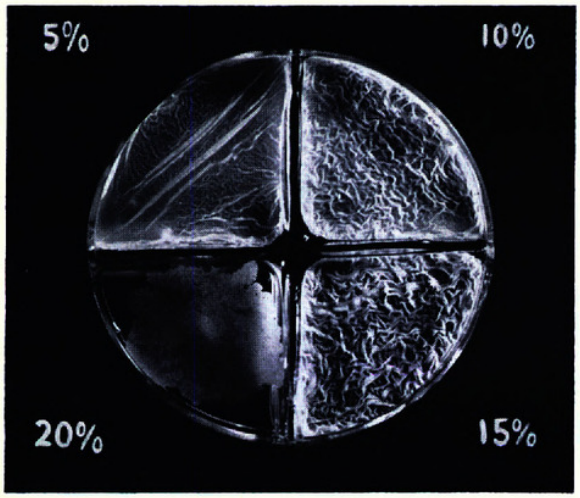
8. Round to oval cells from synthetic agar-A after 7 days. Gram stained, × 1500.



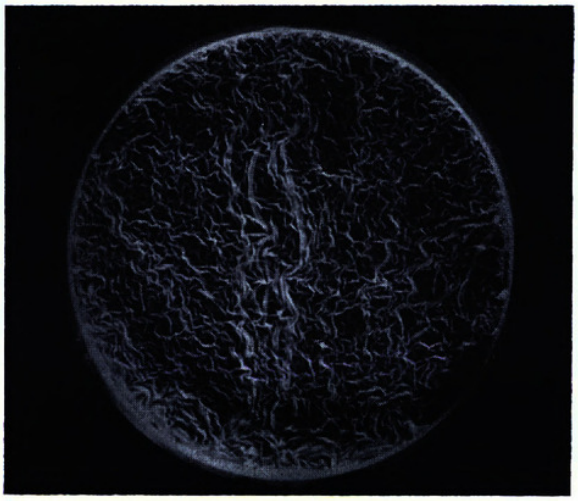
9. Elongated cells from film on 10% salt cucumber brine, 48 hours. Gram stained, × 1500.



10. Cells from cornmeal agar show negative mycelium test at 3 weeks. Unstained, × 950; enlarged, × 2.

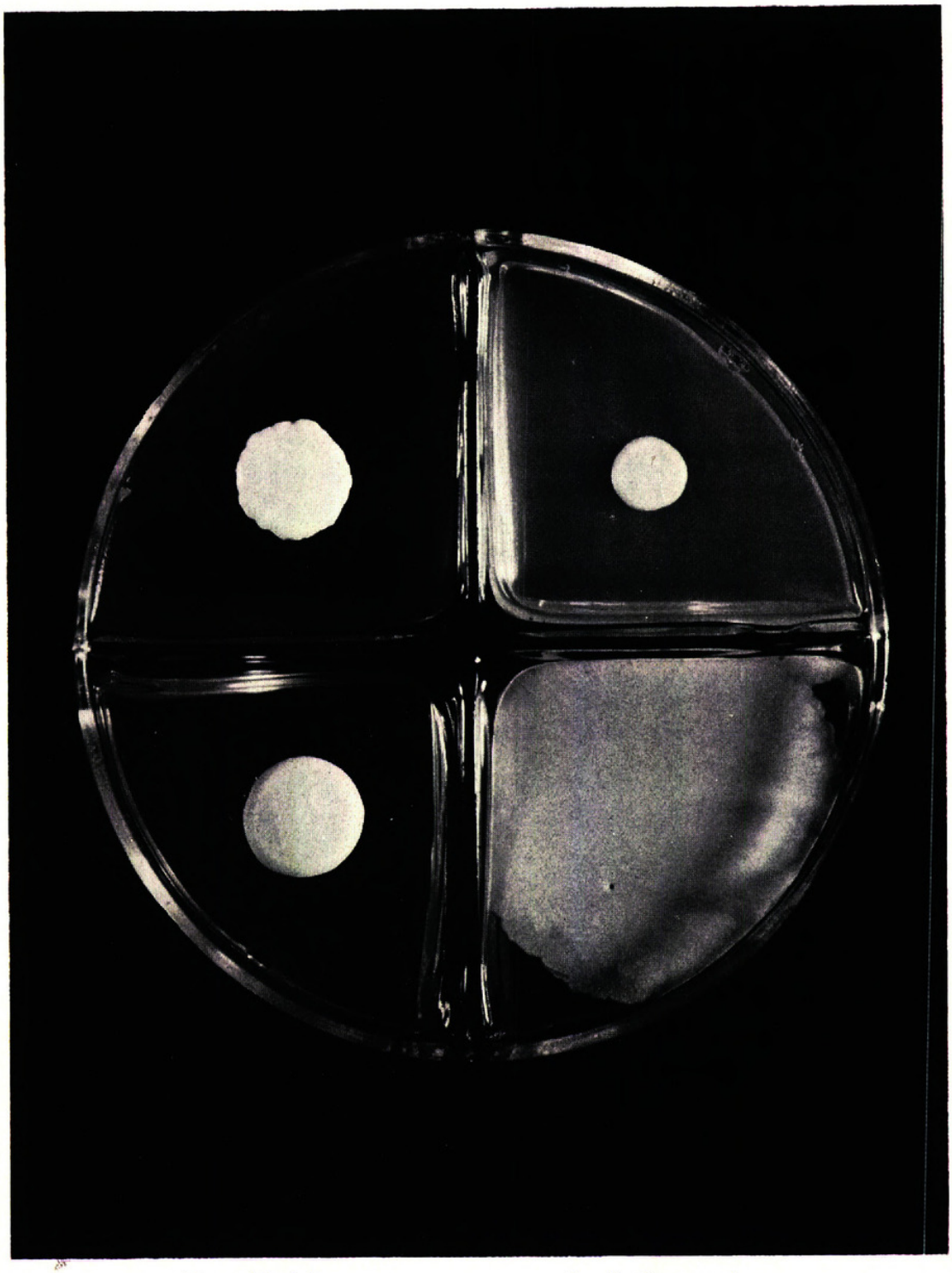


11. Salt-tolerance test at 12 days with films at all 4 brine strengths. × ½.



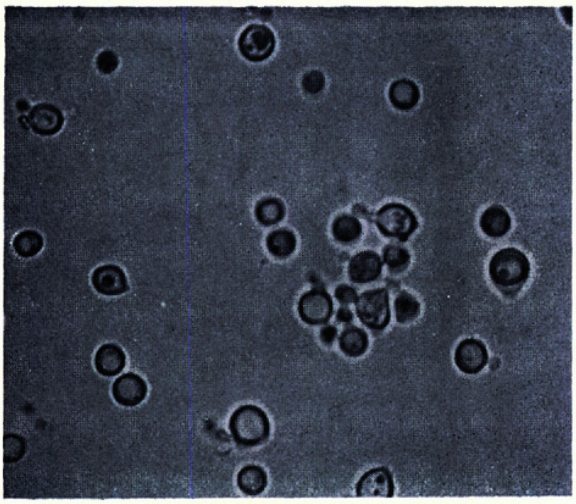
12. Heavy film formation on ethyl alcohol medium at 4 days. × ½.

Debaryomyces

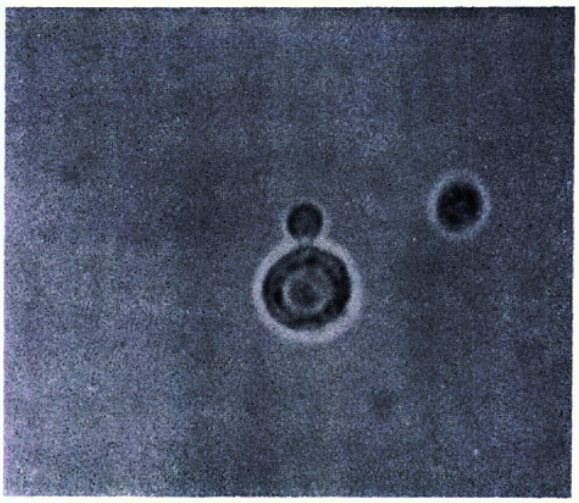


Vegetable-juice agar
Glucose agar
Synthetic agar-A
Glucose-salt agar

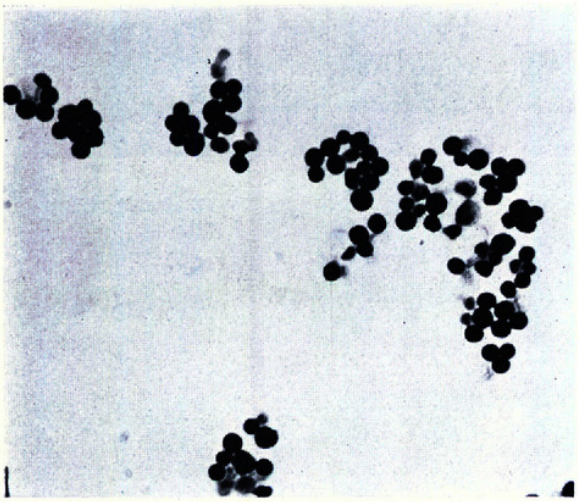
13. Comparative growth by a smooth species of *Debaryomyces* (FY-34) on different cultural media after 6 weeks' incubation at room temperature. Slightly enlarged. SEE OPPOSITE PAGE FOR CELLS AND GROWTH TESTS OF THIS YEAST.



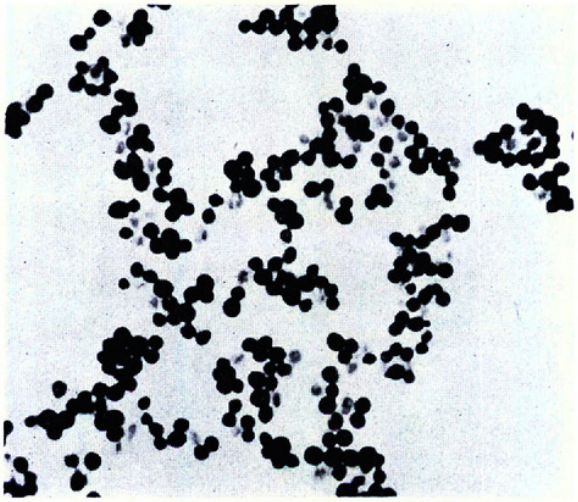
14. Round cells from vegetable-juice agar at 2 months; ascus filled with single spore at right center. Unstained, × 1500.



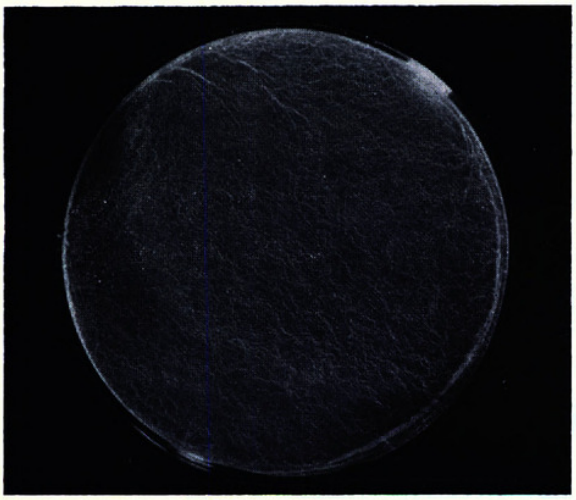
15. Typical ascus with rough spore and centrally located oil drop. From vegetable-juice agar at 2 months. Unstained, × 1500; enlarged, × 2.



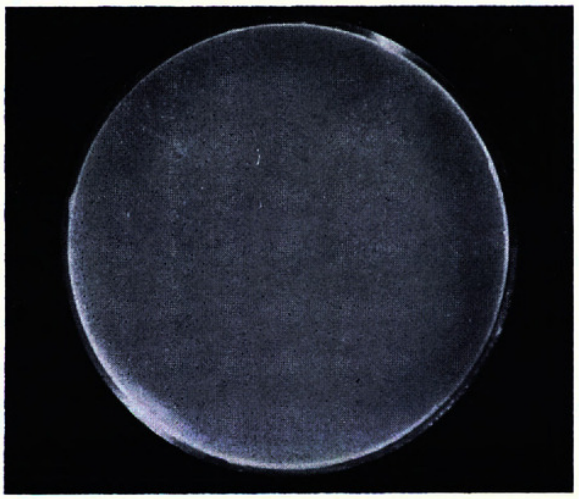
16. Cells from synthetic agar-A after 7 days. Gram stained, × 1500.



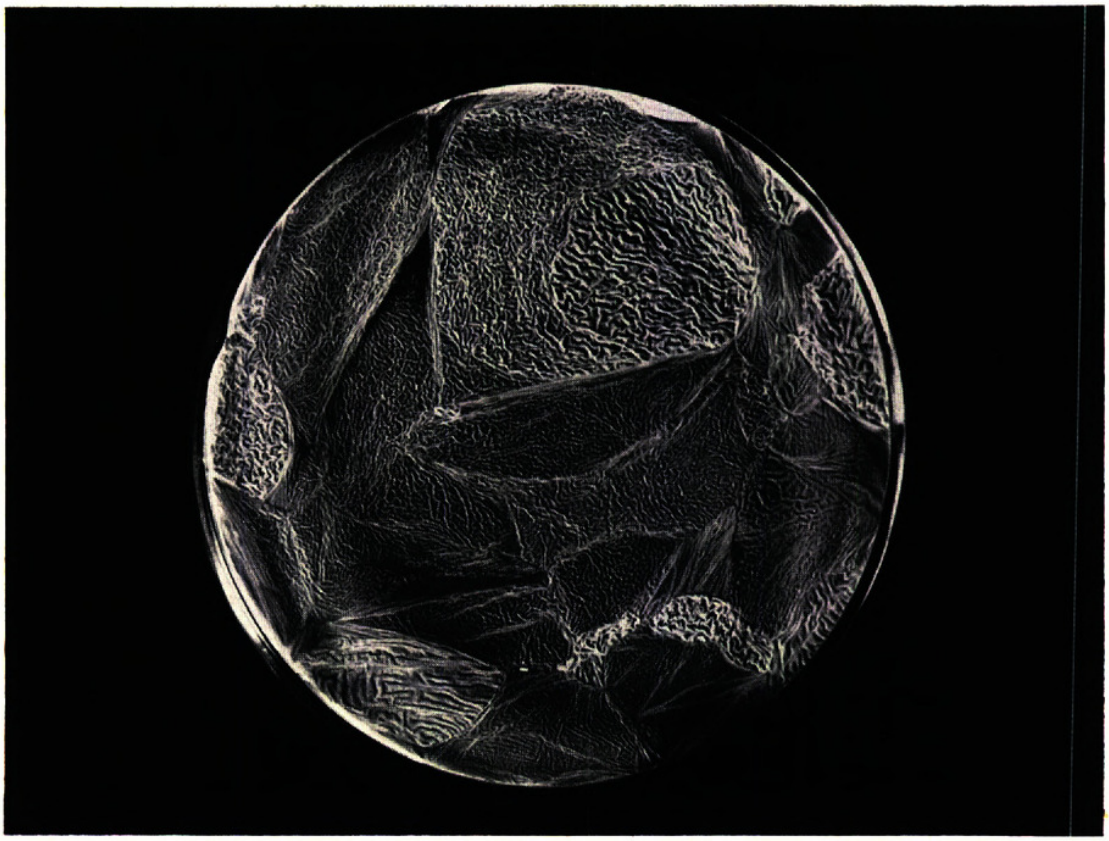
17. Cells from film on 10% salt cucumber brine after 5 days. Gram stained, × 1500.



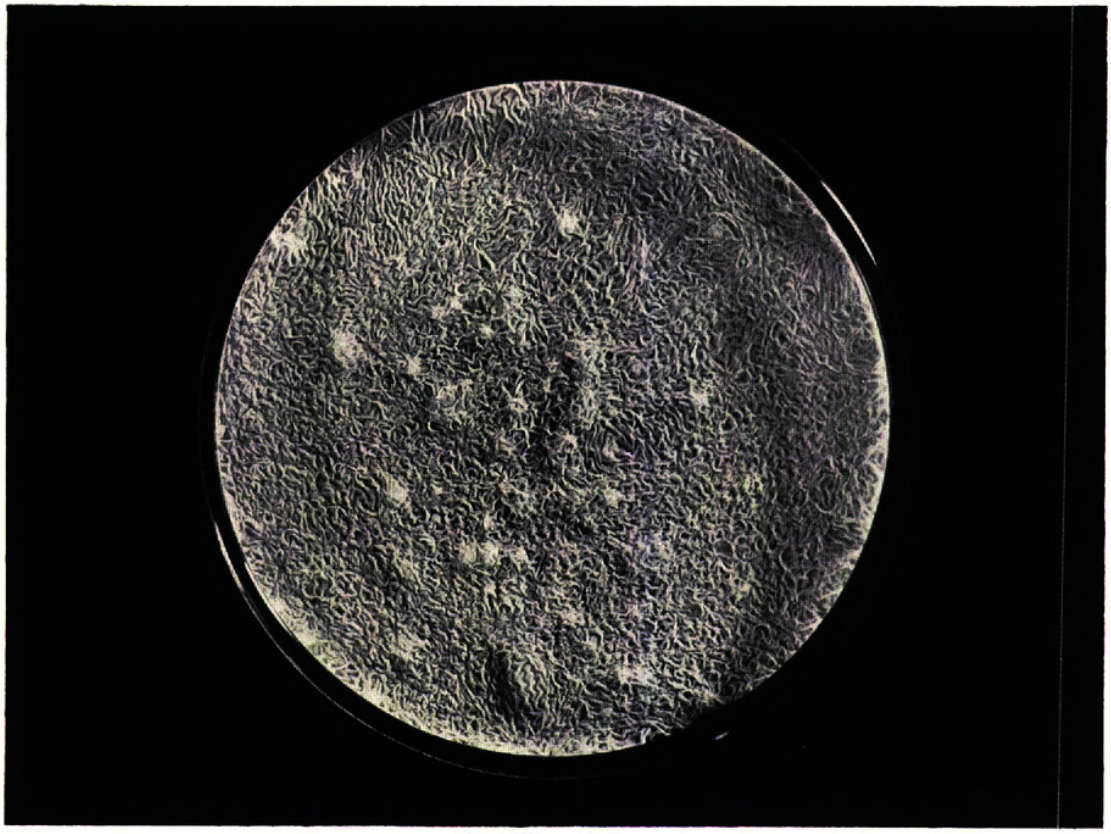
18. Film formation at 3 days on 7% salt cucumber brine. × ½.



19. This yeast forms a very thin film on ethyl alcohol medium. × ½.



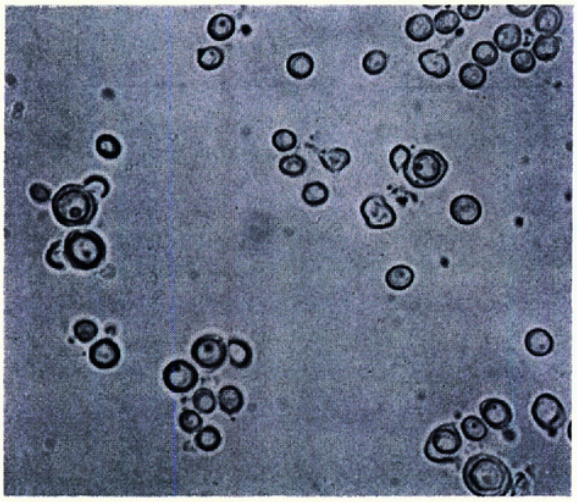
20. Beef tongue brine



21. Ham brine

Naturally occurring films on commercial curing brines from beef tongues (above) and hams (below) after 7 days' incubation at room temperature. Slightly reduced in size. The principal yeast species responsible for these films is the Georgia strain of *D. membranaefaciens* var. *Hollandicus* (shown on pages 268, 269). SEE OPPOSITE PAGE FOR CELLS AND GROWTH TESTS OF SUBSURFACE SPECIES OF *DEBARYOMYCES* FROM BRINED BACON SIDES.

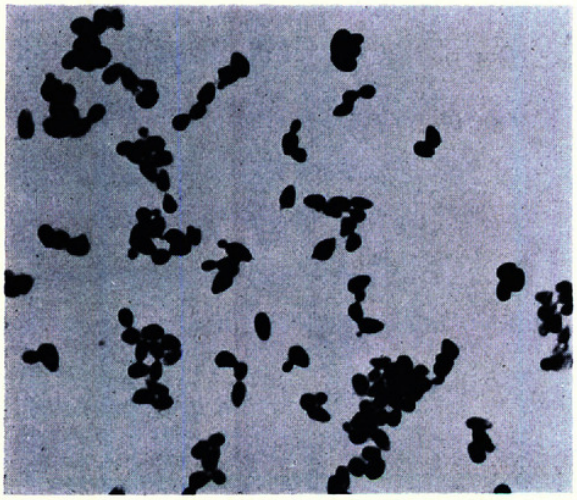
DEBARYOMYCES SP. (Y-6-BA)



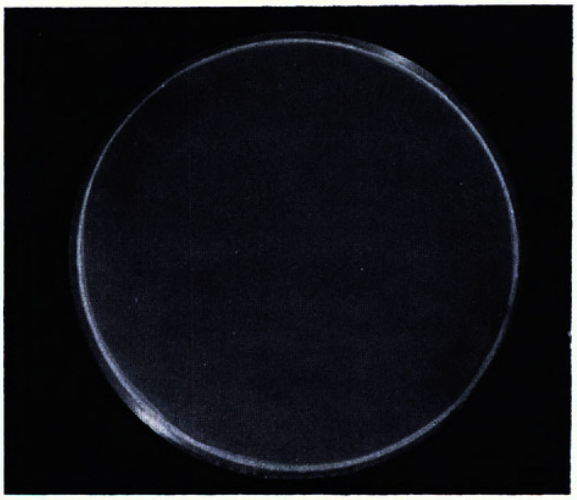
22. Several typical asci with single rough spores; from vegetable-juice agar at 2 months. Unstained, × 1500.

23. Absence of film or subsurface growth in ethyl alcohol medium at 3 days is typical of this species. × ½.

DEBARYOMYCES SP. (Y-37-BA)

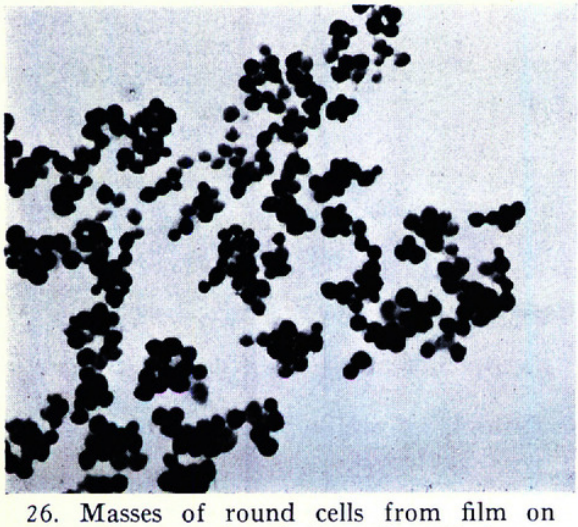


24. Somewhat pointed cells from film on 5% salt cucumber brine after 48 hours. Gram stained, × 1500.

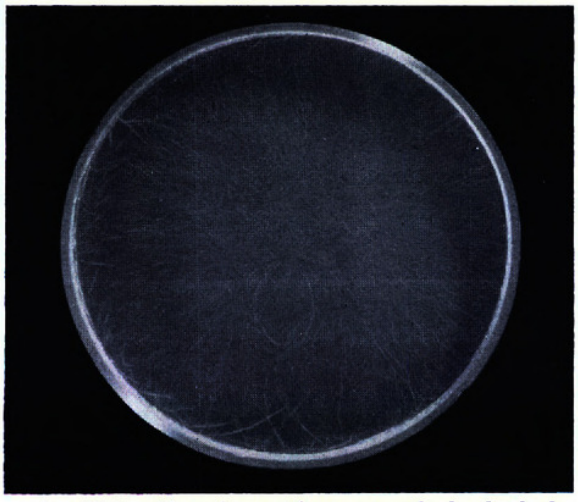


25. Very thin climbing scum is formed by this yeast on ethyl alcohol medium at 3 days. × ½.

DEBARYOMYCES SP. (Y-40-BA)

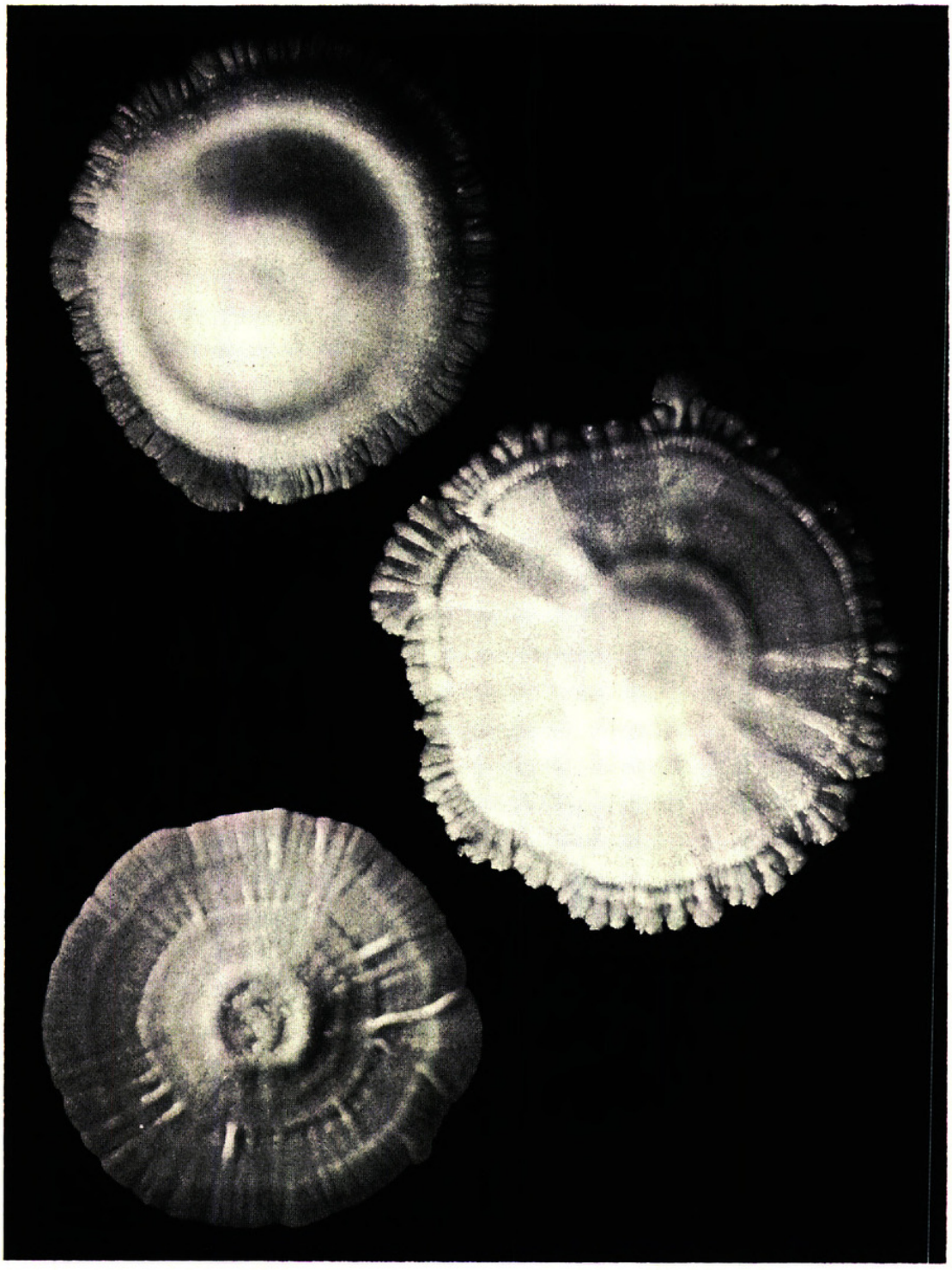


26. Masses of round cells from film on 5% salt cucumber brine after 48 hours. Gram stained, × 1500.



27. Thin film formation on ethyl alcohol medium at 3 days is typical of this species. × ½.

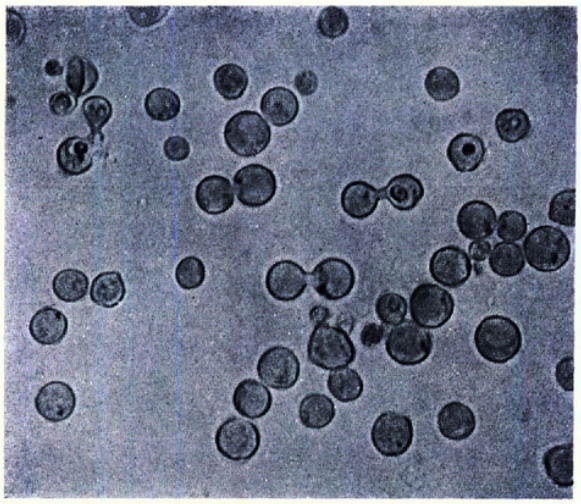
Zygosaccharomyces



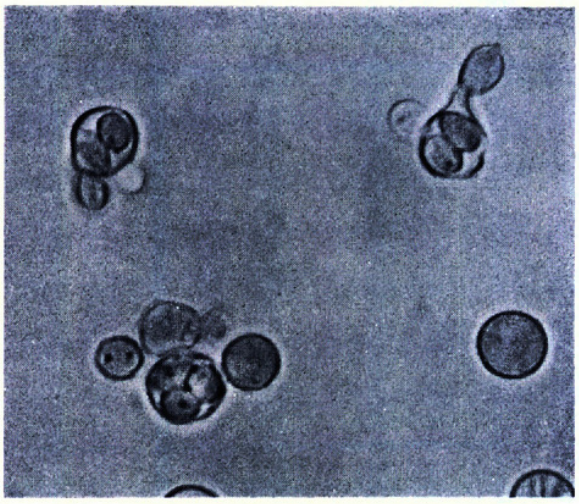
Vegetable-juice agar
Synthetic agar-A

Glucose agar

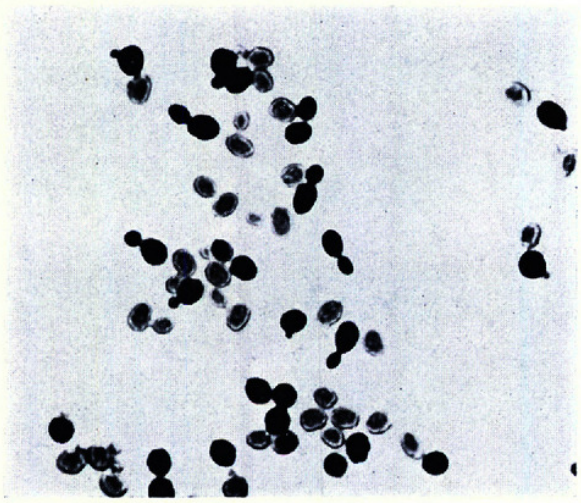
28. Comparative growth by *Zygosaccharomyces halomembranis* Etchells & Bell (Y-1000) on different cultural media after 6 weeks' incubation at room temperature. Colonies enlarged, × 3. In cucumber brines from Michigan, Wisconsin and Indiana, this species occurs both as a surface and subsurface yeast. SEE OPPOSITE PAGE FOR CELLS AND GROWTH TESTS OF THIS YEAST.



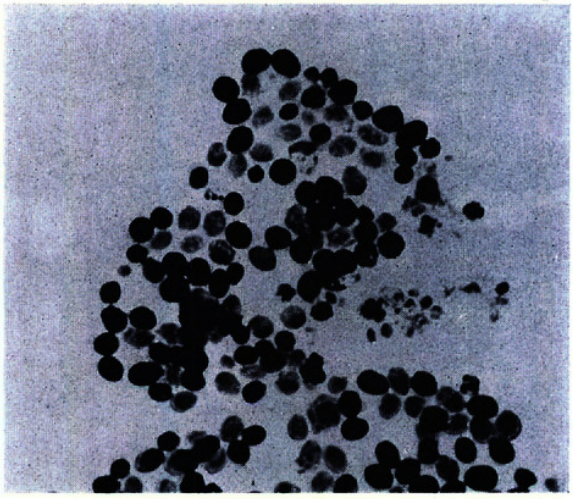
29. Early stage of sporulation, with conjugated round cells; from vegetable-juice agar at 3 weeks. Unstained, × 1500.



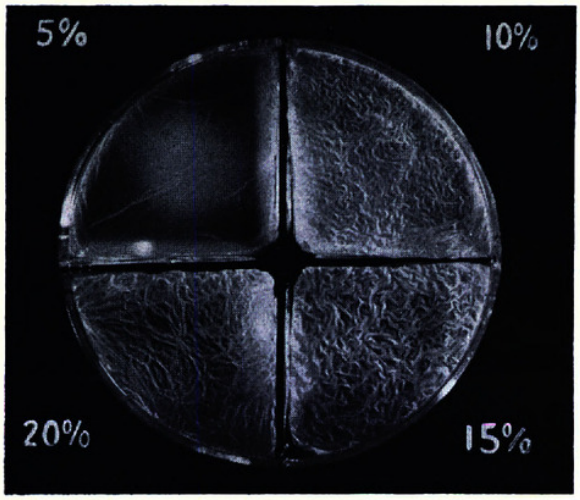
30. Fully developed asci with 2 and 3 oval spores each; from vegetable-juice agar at 1 month. Unstained, × 1500; enlarged, × 2.



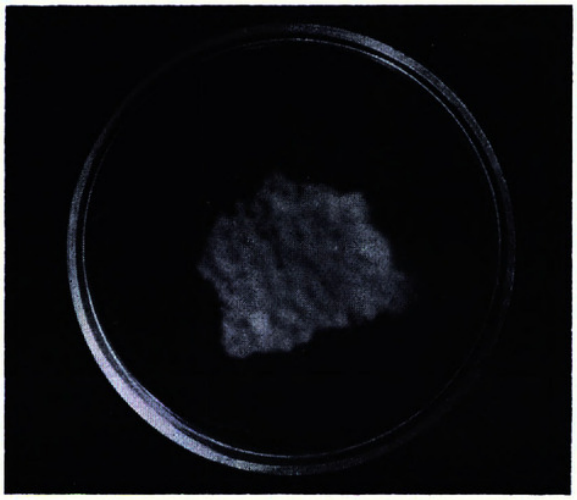
31. Young cells, 48 hours old, from film on 10% salt cucumber brine. Gram stained, × 1500.



32. Masses of older cells, 5 days old, from film on 10% salt cucumber brine. Gram stained, × 1500.

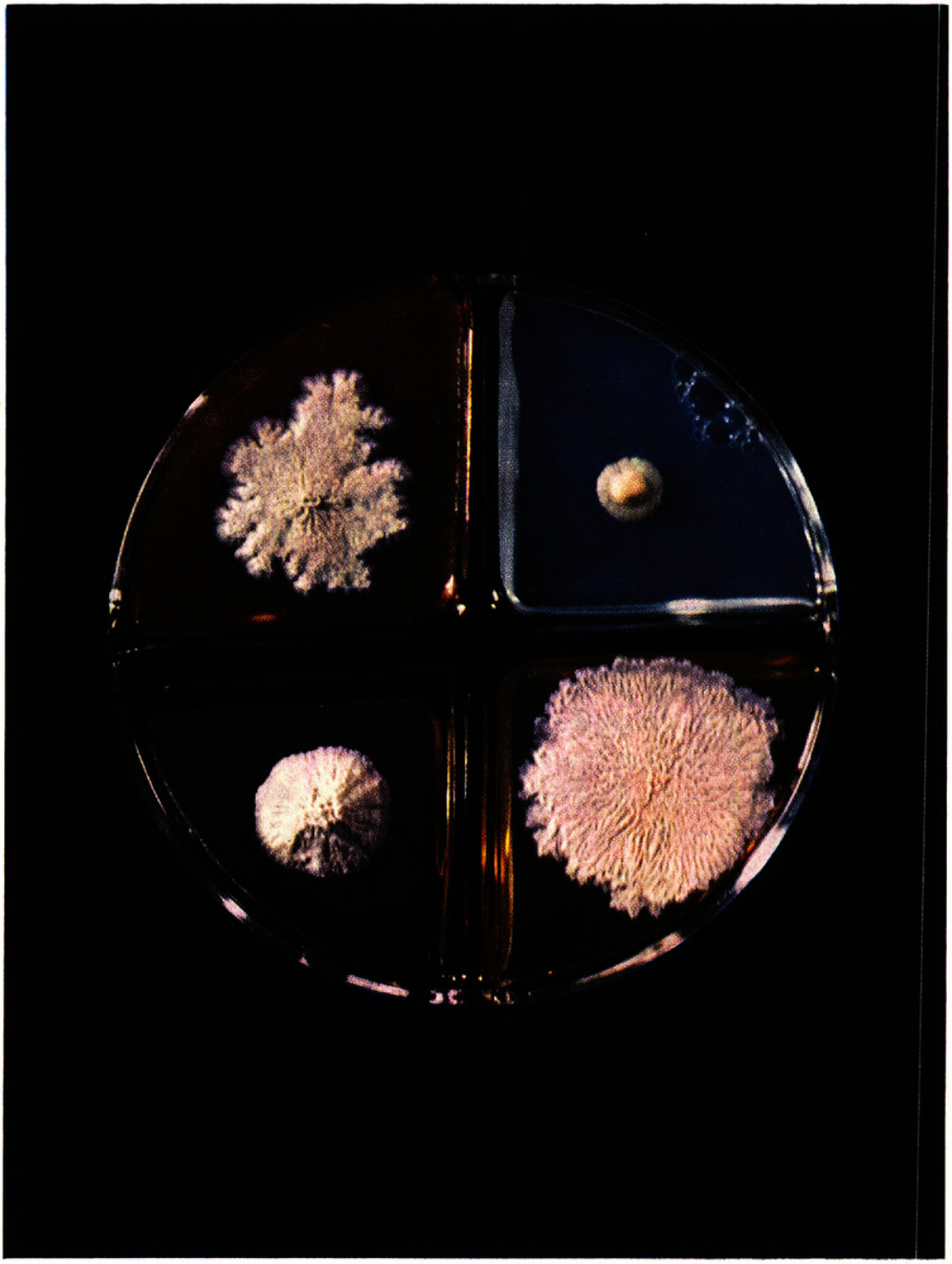


33. Salt-tolerance test at 5 days shows heavy film formation at all 4 salt concentrations. × ½.



34. Subsurface growth but no film formation on ethyl alcohol medium is typical for this species. × ½.

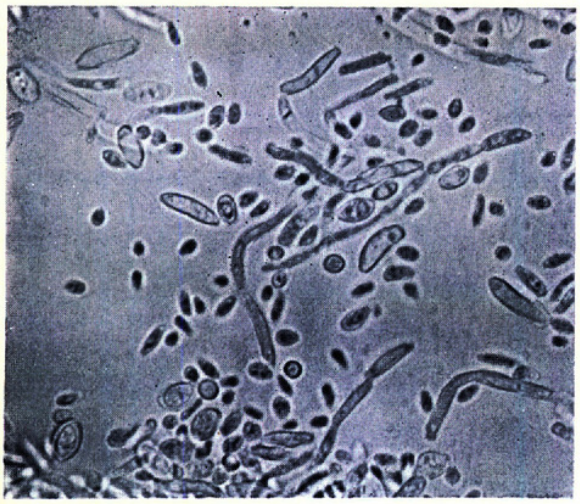
Endomycopsis



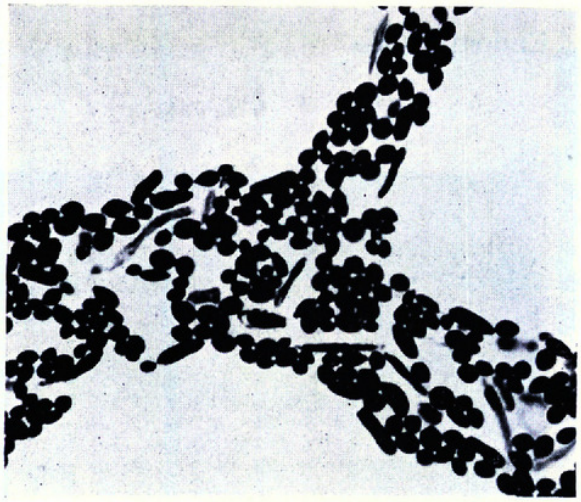
Vegetable-juice agar
Glucose agar

Synthetic agar-A
Glucose-salt agar

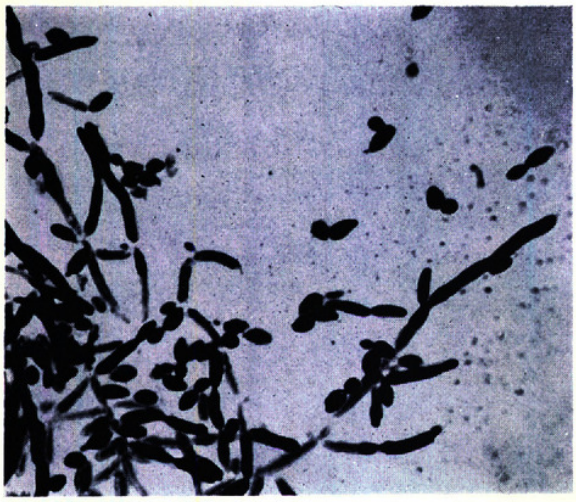
C. Comparative growth by *Endomycopsis ohmeri* Etchells & Bell (FY-25) on different cultural media after 6 weeks' incubation at room temperature. Actual size. So far this species has only been isolated from North Carolina brines. SEE OPPOSITE PAGE FOR CELLS AND GROWTH TESTS OF THIS YEAST.



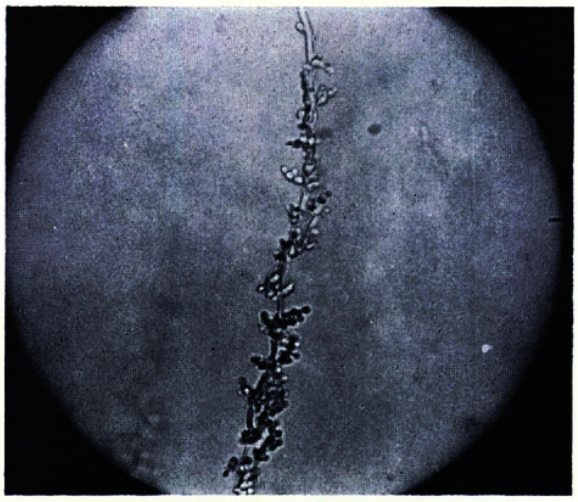
35. Pleomorphic cells from vegetable-juice agar after 4 months. No spores present. Unstained, × 1500.



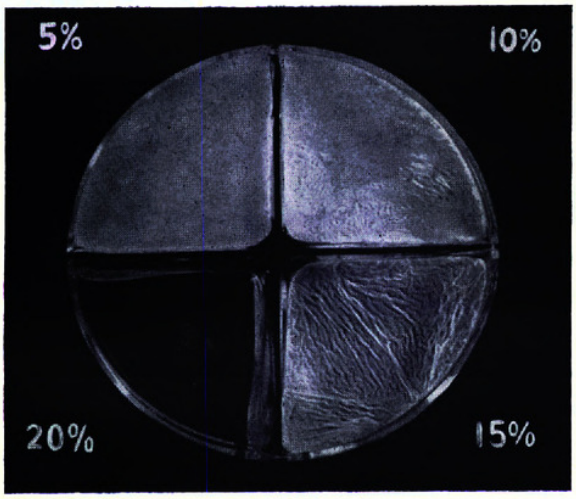
36. Typical cells from synthetic agar-A after 7 days. Gram stained, × 1500.



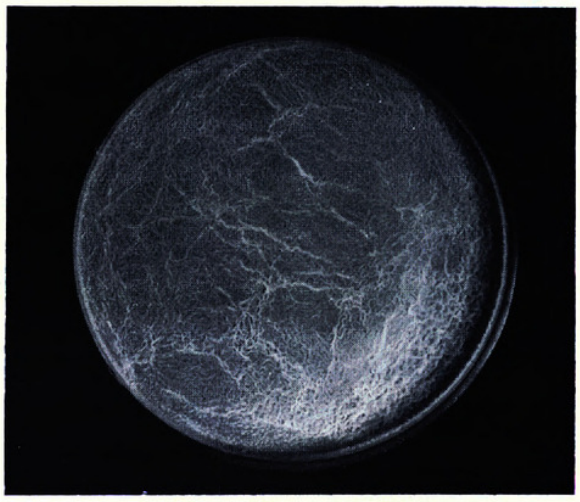
37. Elongated cells from film on 10% salt brine after 48 hours. Gram stained, × 1500.



38. Single, long mycelial thread with clusters of cells; from cornmeal agar after 3 weeks. Unstained, × 650.

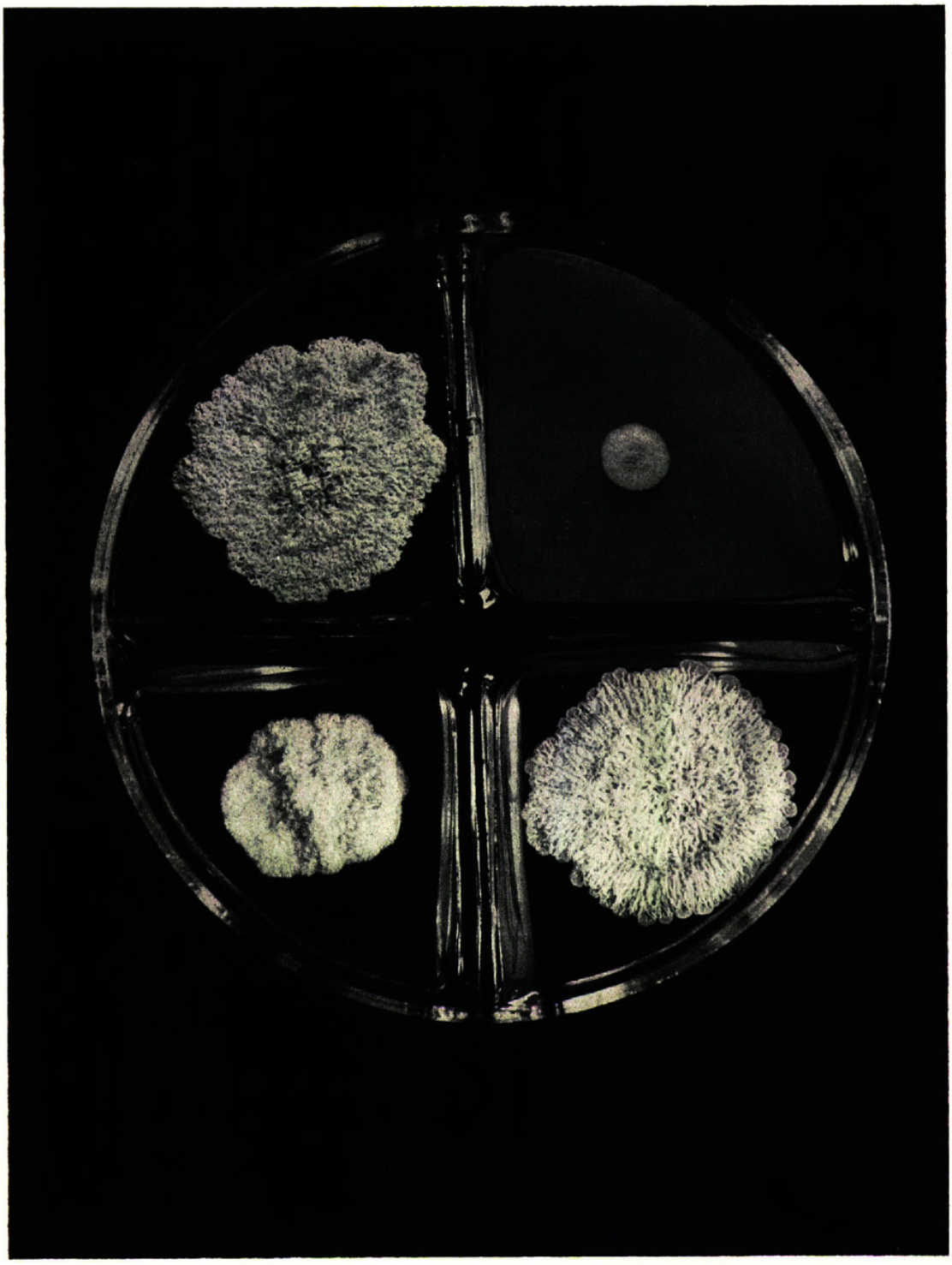


39. Salt-tolerance test shows film growth on 15% brine but not 20% at 5 days. Films disintegrate quickly at 5 and 10%. × ½.



40. Good film growth occurs in 4 days on ethyl alcohol medium. × ½.

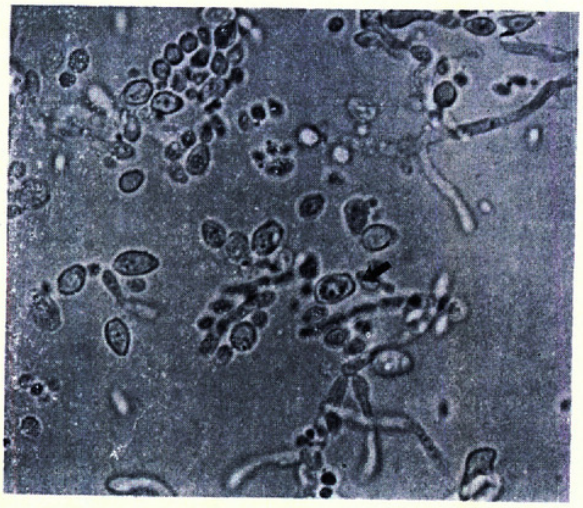
Endomycopsis



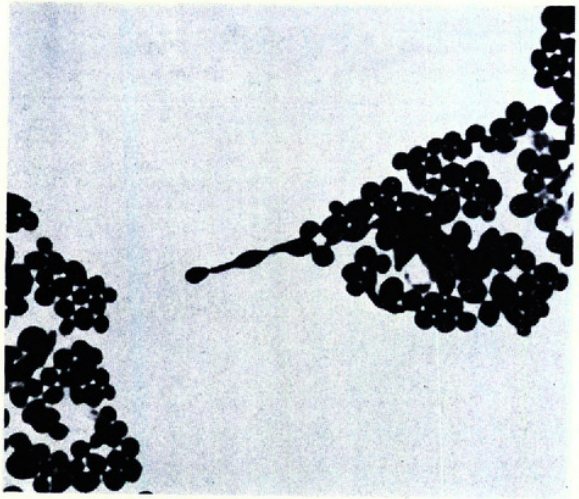
Vegetable-juice agar
Glucose agar

Synthetic agar-A
Glucose-salt agar

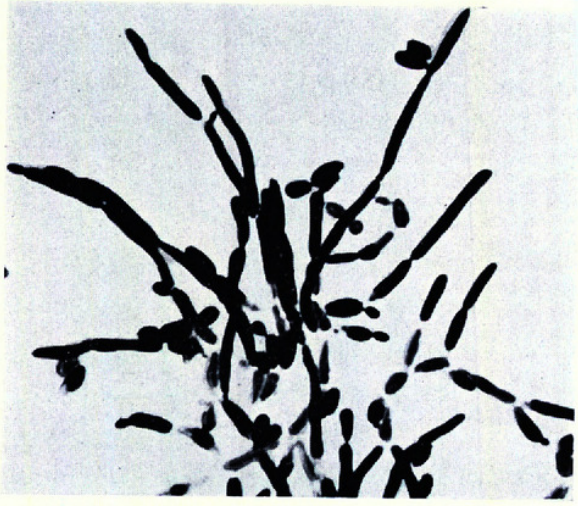
41. Comparative growth by *Endomycopsis ohmeri* var. *minor* Etchells & Bell (FY-1) on different cultural media after 6 weeks' incubation at room temperature. Slightly enlarged. SEE OPPOSITE PAGE FOR CELLS AND GROWTH TESTS OF THIS YEAST.



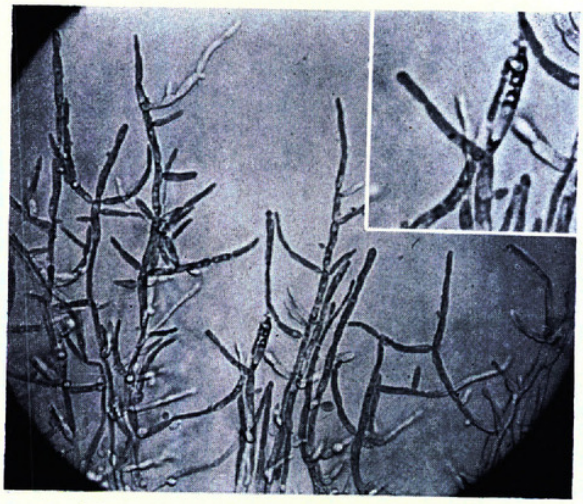
42. Pleomorphic cells from vegetable-juice agar after 4 months. Single spore in ascus (arrow). Unstained, × 1500.



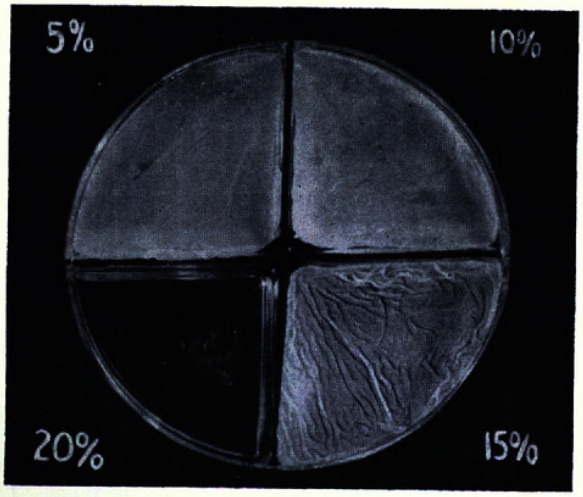
43. Cells from synthetic agar-A after 7 days. Gram stained, × 1500.



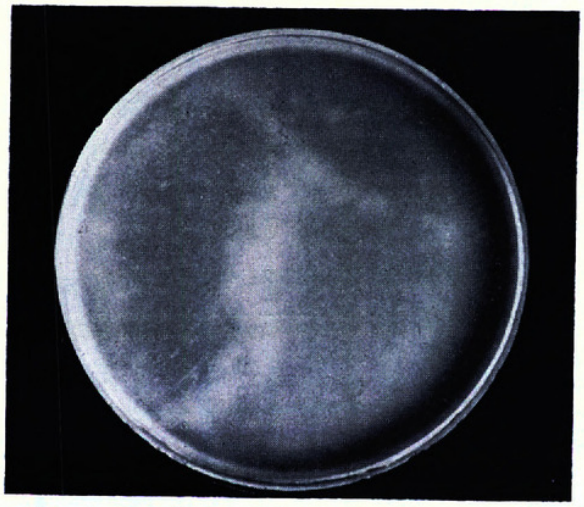
44. Elongated cells from film on 10% salt brine after 48 hours. Gram stained, × 1500.



45. Mycelium formation on cornmeal agar after 3 weeks; unstained, × 650. Insert, 2 spores from mycelium, enlarged, × 2.

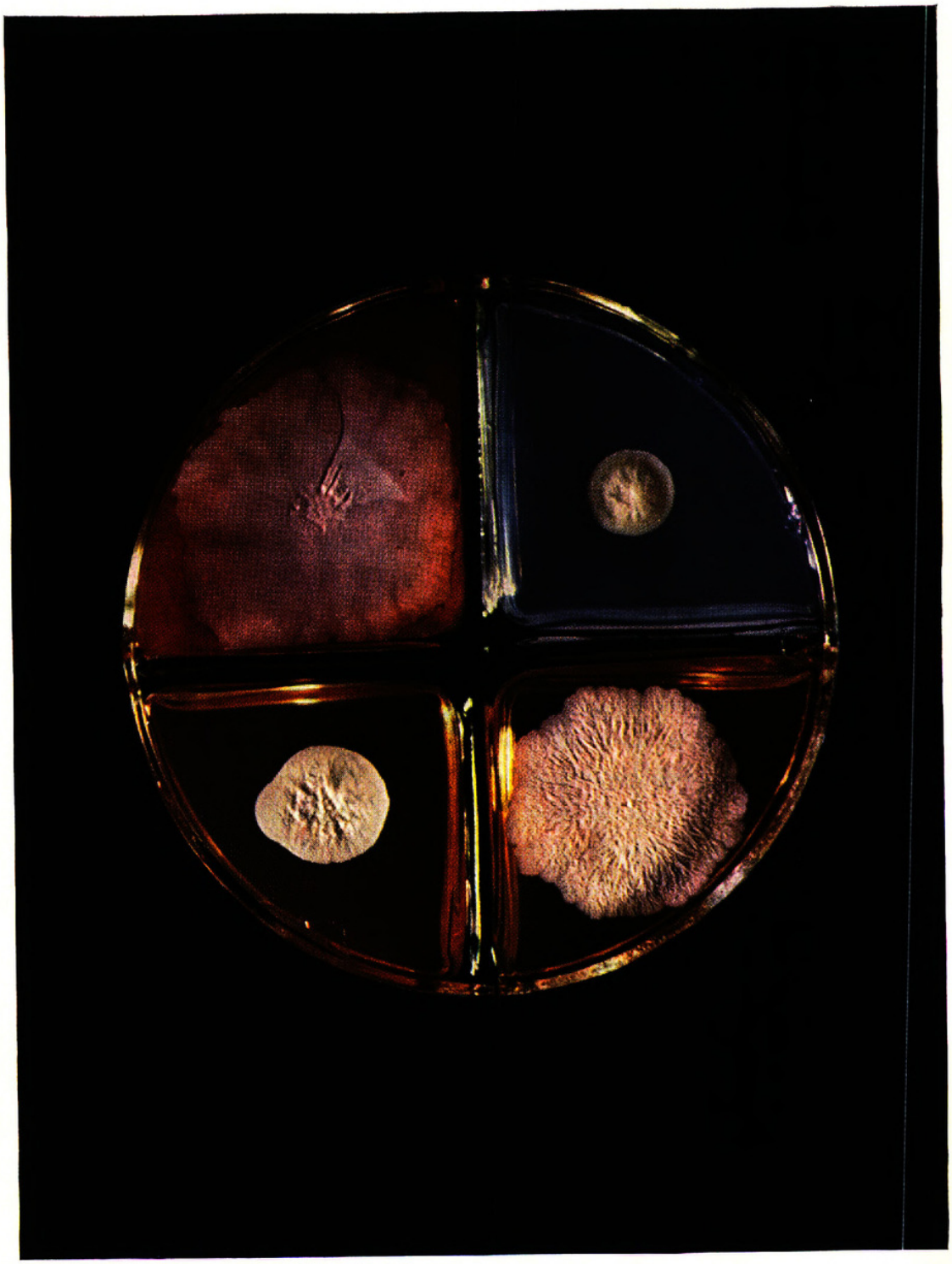


46. Salt-tolerance test shows film growth on 15% brine but not 20% at 5 days. Films on 5 and 10% have fallen. × ½.



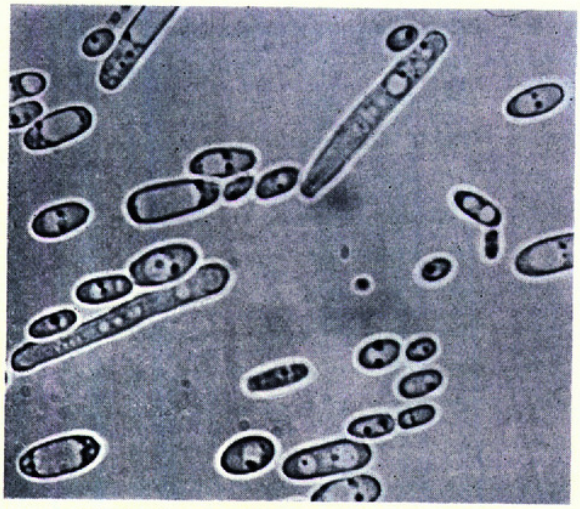
47. A smooth, membrane-type film forms on ethyl alcohol medium in 4 days. × ½.

Candida

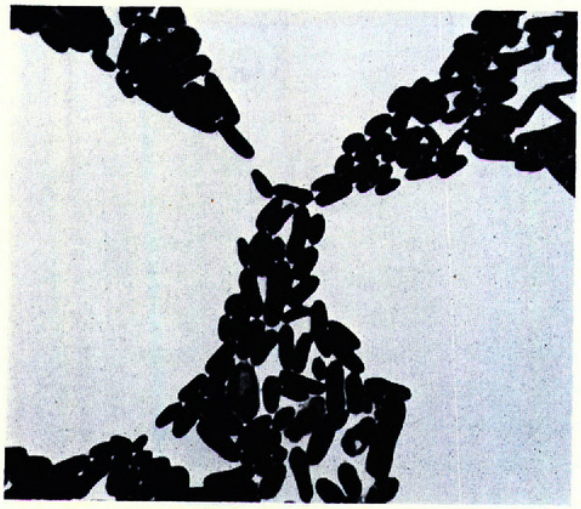


Vegetable-juice agar
Glucose agar
Synthetic agar-A
Glucose-salt agar

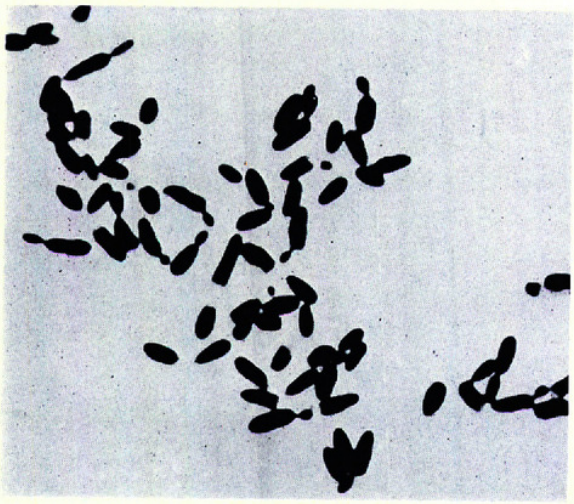
D. Comparative growth by *Candida krusei* (A. Cast.) Berkhout (FY-20) on different cultural media after 6 weeks' incubation at room temperature. Actual size. SEE OPPOSITE PAGE FOR CELLS AND GROWTH TESTS OF THIS YEAST.



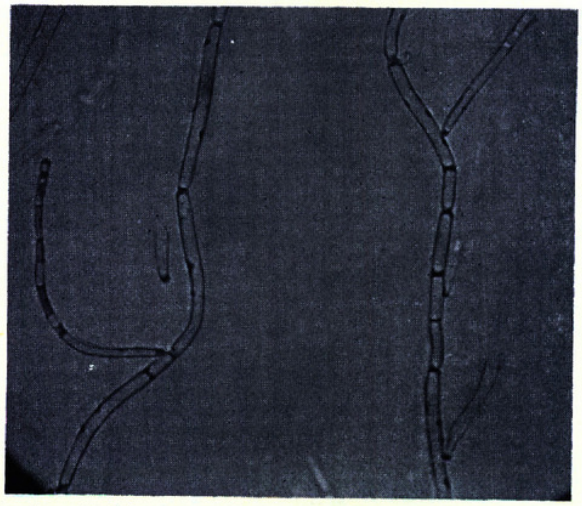
48. Typical cells from vegetable-juice agar at 3 weeks. Unstained, × 1500.



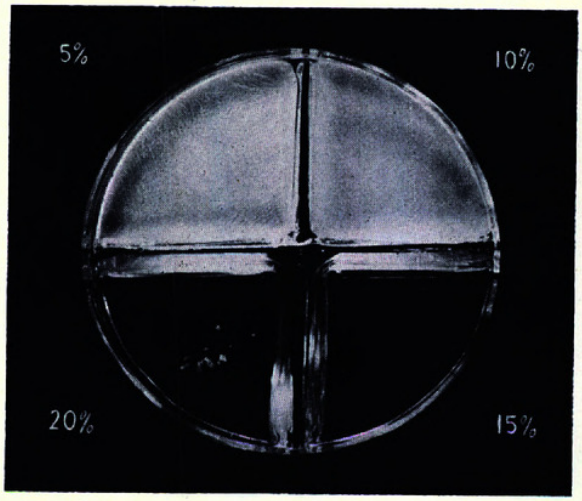
49. Cells from synthetic agar-A after 7 days. Gram stained, × 1500.



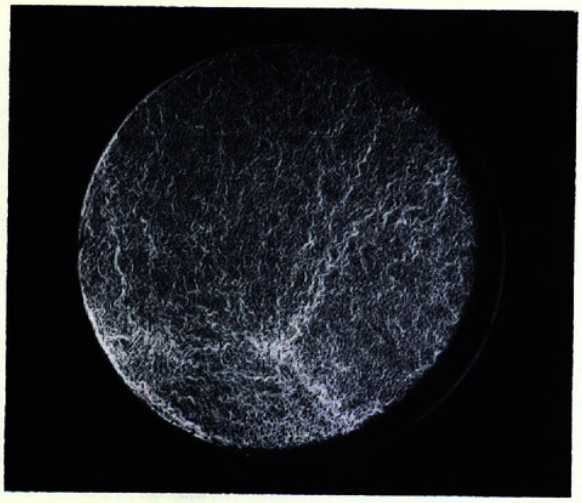
50. Cells from film on 10% salt brine after 5 days. Gram stained, × 1500.



51. Evidence of septated mycelium on cornmeal agar after 3 weeks. Unstained, × 650; enlarged, × 2.

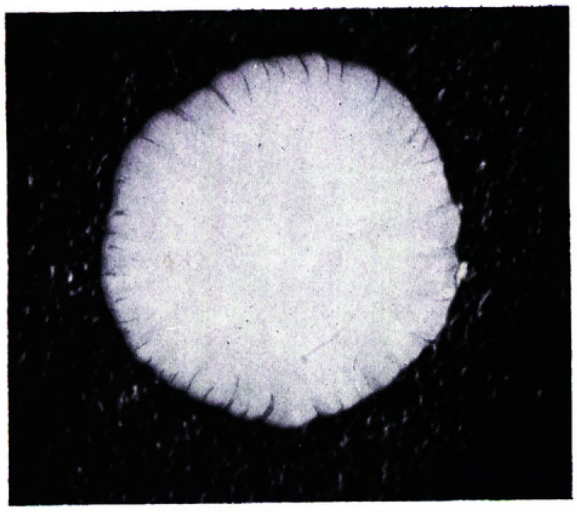


52. Salt-tolerance test shows no film growth above 10% brine strength after 7 days. × ½.

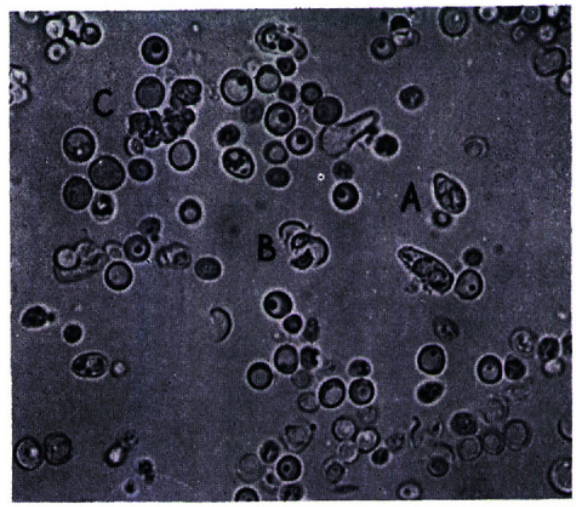


53. Heavy film formation on ethyl alcohol medium at 4 days. × ½.

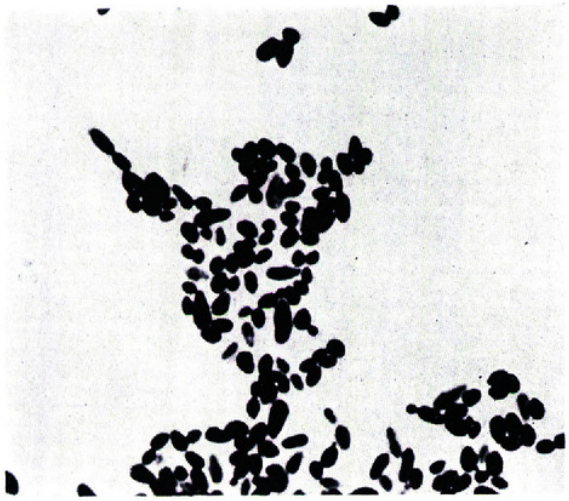
HANSENULA ANOMALA (HANSEN) SYDOW (KS)



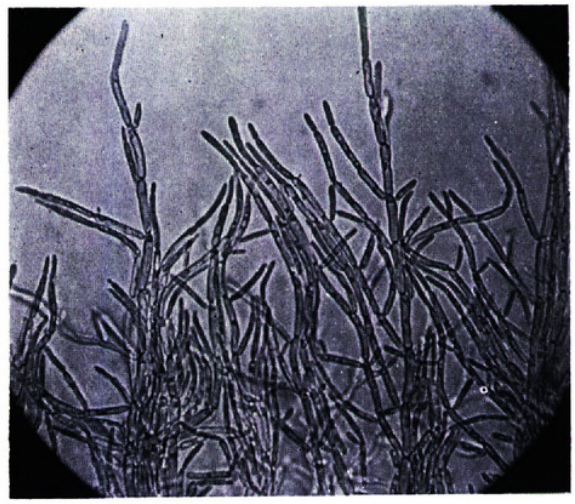
54. Giant colony grown on vegetable-juice agar, 6 weeks. × 2.



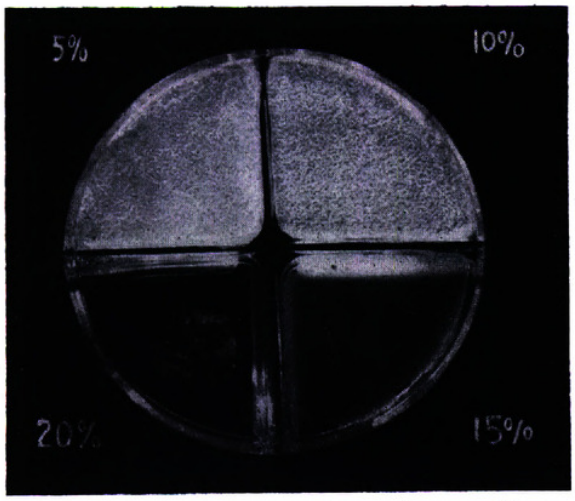
55. Sporulation at 2 months. *A*, hat-shaped spores in ascus; *B*, emerging spore; *C*, cluster of free spores. Unstained, × 1500.



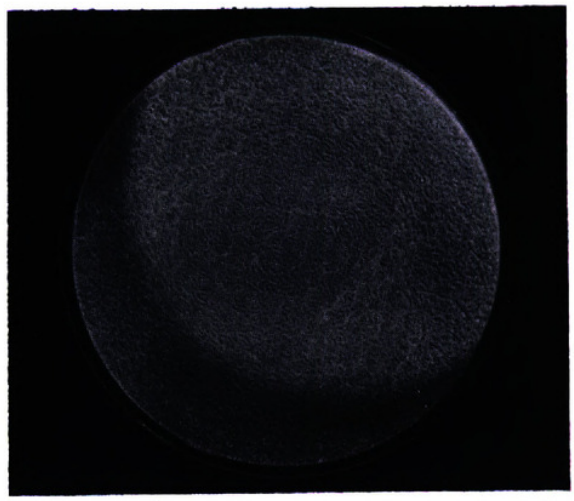
56. Cells from film on 5% salt brine after 48 hours. Gram stained, × 1500.



57. Mycelium formation on cornmeal agar after 3 weeks. Unstained, × 650.

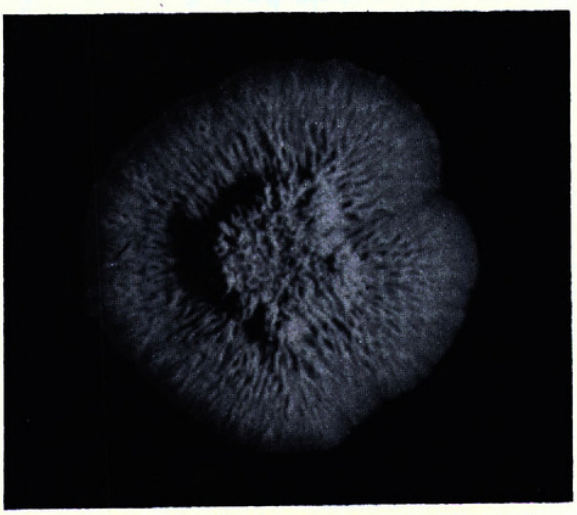


58. Salt-tolerance test shows heavy films at 5 and 10% brines, a very thin film on 15%, after 7 days. × ½.

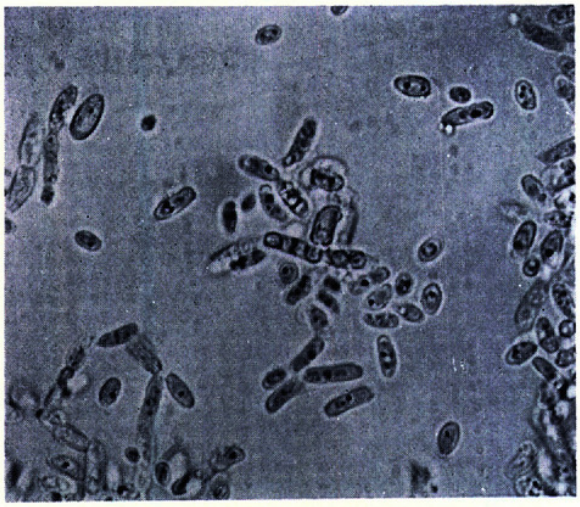


59. Heavy film formation on ethyl alcohol medium at 4 days. × ½.

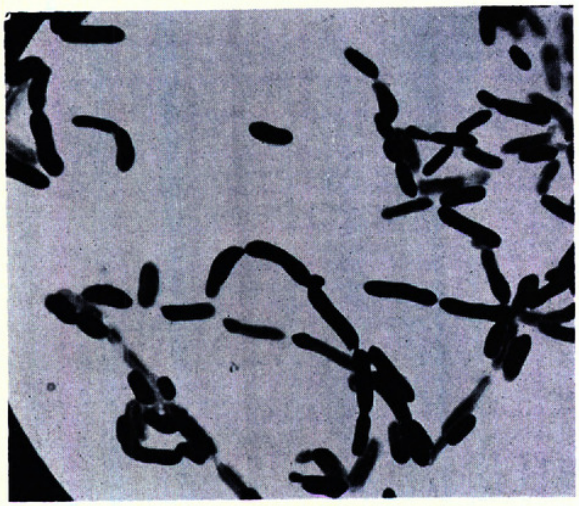
PICHIA ALCOHOLOPHILA KLOCKER (FY-31)



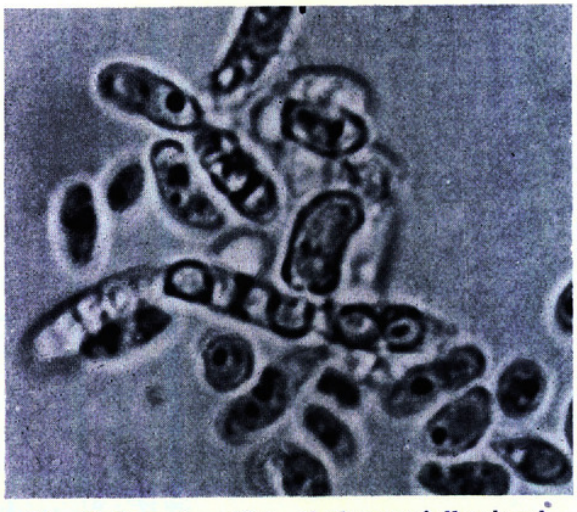
60. Giant colony grown on vegetable-juice agar; at 6 weeks. × 2.



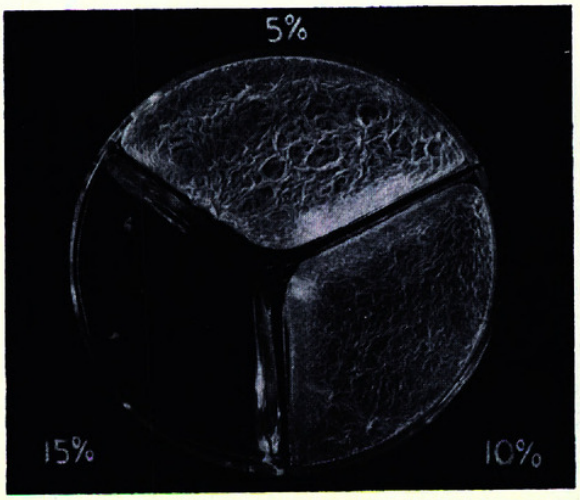
61. Sporulation on vegetable-juice agar at 2 months. Unstained, × 1500.



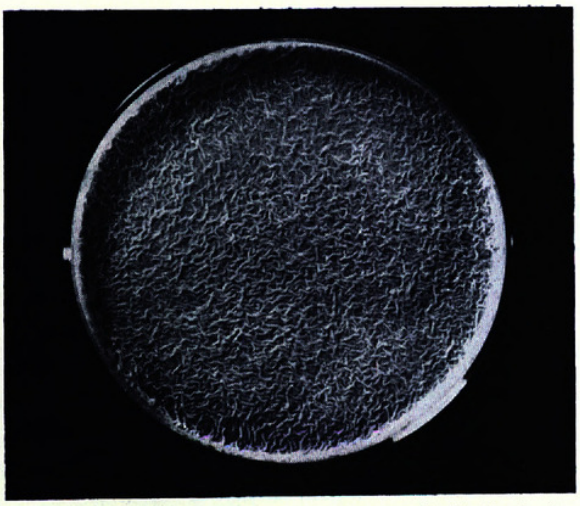
62. Sausage-shaped cells from film on 5% saltbrine after 48 hours. Gram stained, × 1500.



63. Enlarged section of above; fully developed asci with 2 and 4 helmet-shaped spores.



64. Salt-tolerance test shows film growth at 5 and 10% brines; no growth at 15% after 3 days. × ½.



65. Heavy film formation on ethyl alcohol medium at 4 days. × ½.

SUBSURFACE BRINE YEASTS

Active growth by the fermentative subsurface yeasts in commercial cucumber brines was first reported in 1941 *(5)*. Since then, their growth activity has been reported in a variety of other brined and salted vegetables *(12)*. In contrast to film-forming types, the growth of subsurface species in brines is characterized by a gaseous fermentation which results in the evolution of rather large amounts of carbon dioxide.

Further, their growth covers a wide range with respect to brine concentration, the maximum salt-tolerance observed under commercial conditions being in the neighborhood of 20 to 22 percent by weight (saturation being 26.4). Usually, the salt content of the brine determines the time yeast growth starts as well as the duration of activity.

As a rule, fermentations in low salt content brines (about 5 percent) start earlier and are of shorter duration than those at higher concentrations (10 to 15 percent). The reason for more active yeast development in the stronger brines is that the lactic acid bacteria are inhibited as the brine strength increases and more food material remains for the yeasts which are much more salt-tolerant.

Studies on commercially brined cucumbers represent the principal source of information on the identity and sequence of individual yeast species in brine-fermented foods. Basic investigations of this type have been reported in detail *(6, 11)* for the two major cucumber brining areas in the country — northern and southern. A brief summary of these two studies, based on the identity of nearly 1,900 cultures, demonstrates that the pattern for the principal yeast species in brines from both areas is very similar. Seven of the nine species found were obtained from both northern and southern brines *(i.e. Brettanomyces versatilis, Torulopsis caroliniana, Torulopsis holmii, Torulaspora rosei, Hansenula subpelliculosa, Zygosaccharomyces halomembranis* and *Zygosaccharomyces globiformis)*. The presence of *Saccharomyces globosus* in northern brines was considered to be the principal floral difference.

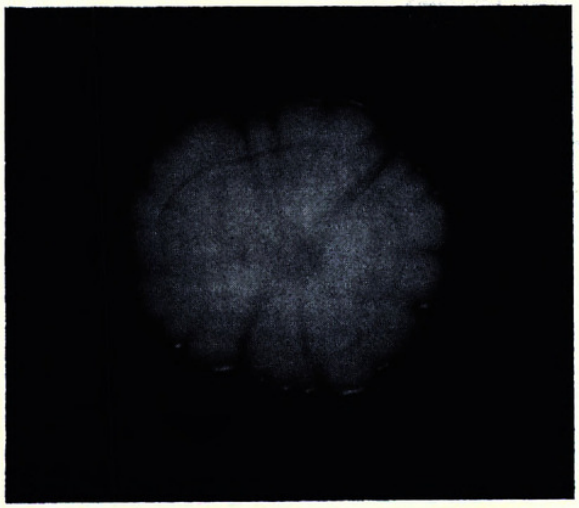
Because a gaseous fermentation by subsurface yeasts is associated with a type of spoilage known as "bloater" or hollow cucumber formation, yeast growth is of economic importance to the pickling industry. "Bloating" can be either in the form of lens-shaped gas pockets in the tissue, or the gas pressure can be sufficient to press the whole seed portion of the cucumber toward the skin, thus leaving a large gas-filled cavity.

Yeasts are also responsible for certain types of spoilage in manufactured pickle products. This is particularly true in cases of non-pasteurized products where the vinegar and sugar concentrations are insufficient to inhibit their growth, or, where they are allowed to develop high tolerance to vinegar and sugar through lack of plant sanitation. In a recent outbreak of spoilage of sweet pickles a number of very acid- and sugar-tolerant yeast cultures were obtained and identified as *Zygosaccharomyces globiformis* (*2*). It is recalled that this yeast was found in cucumber fermentations located in the principal brining areas of the country.

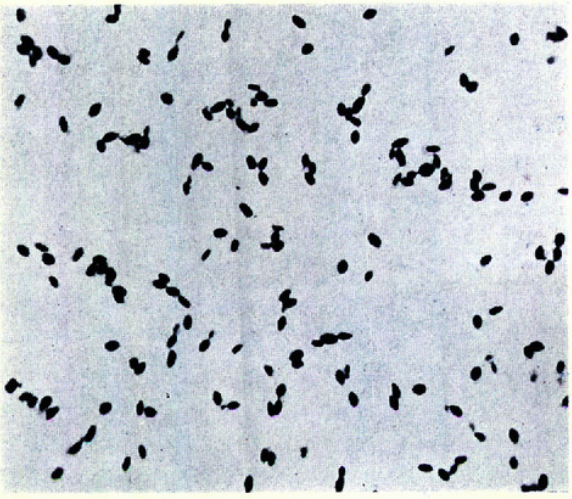
So far, the brine yeast species studied have not been incriminated as a potential source of the cucumber salt-stock softening enzyme pectinase *(8)*. However, a strain of *S. cerevisiae* isolated from soft dill pickles (YD-15, page 293) appears to be identical taxonomically with strains isolated from spoiled citrus concentrate (*13*) that do produce this enzyme (D-6, page 293).

Torulopsis

T. CAROLINIANA ETCHELLS & BELL (RY–165)



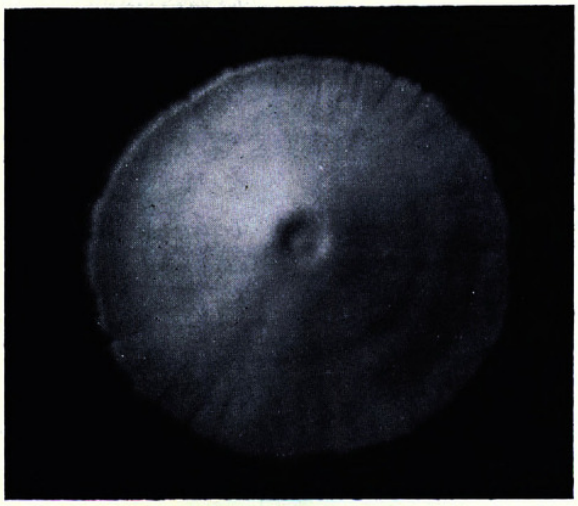
66. Giant colony grown on vegetable-juice agar; at 6 weeks. × 3.



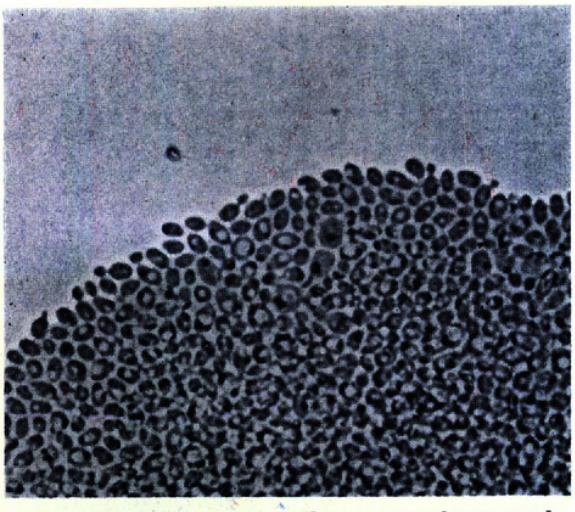
67. Cells from vegetable-juice agar at 1 month. These cells are among the smallest of known yeasts. Gram stained, × 1500.

68. Giant colony grown on synthetic agar-A; at 6 weeks. × 4.

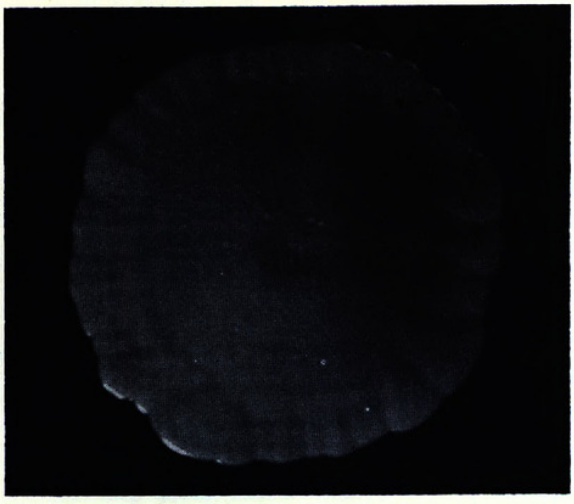
T. HOLMII (JORGENSEN) LODDER (Y–600)



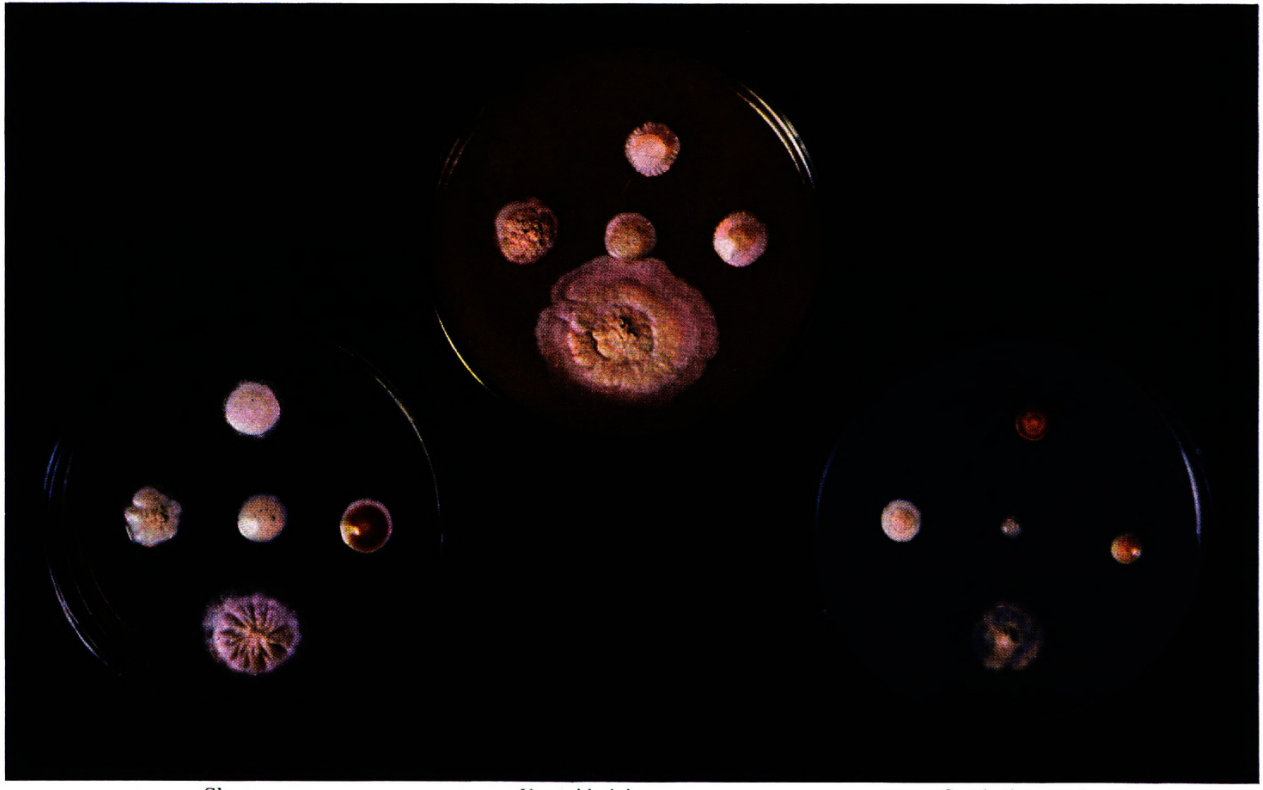
69. Giant colony grown on vegetable-juice agar; at 6 weeks. × 2.



70. Cells from cornmeal agar are short-oval with no tendency to elongate. Unstained, × 950; enlarged, × 2.



71. Giant colony grown on synthetic agar-A; at 6 weeks. × 3.



Glucose agar Vegetable-juice agar Synthetic agar-A

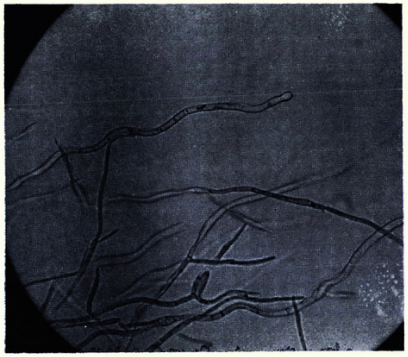
E. Comparative growth of 5 species of *Brettanomyces* on 3 cultural media after 6 weeks' incubation at room temperature. About ⅔ actual size.

↑

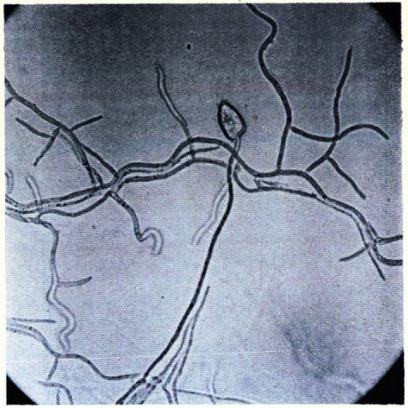
B. bruxellensis

B. lambicus *B. versatilis* *B. claussenii*

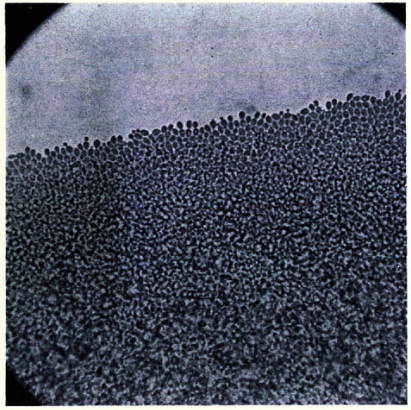
B. anomalus



75. *B. lambicus*

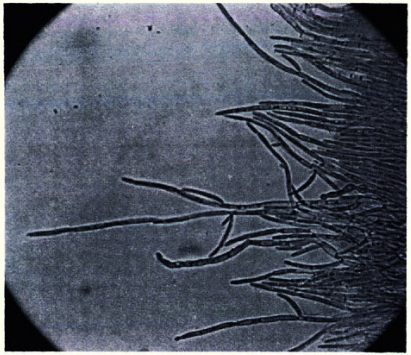


76. *B. anomalus*

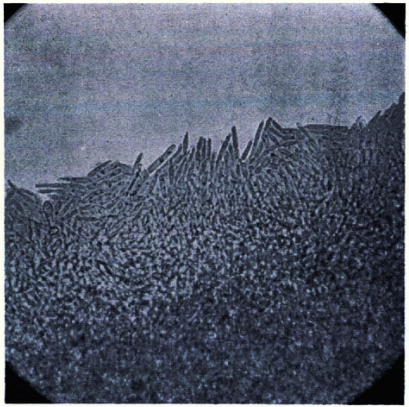


74. *B. versatilis*

Cornmeal agar mycelium tests at 3 weeks for five species of *Brettanomyces*. *B. versatilis* and *B. claussenii* show normal cells; other 3 species produce mycelium. Unstained, × 950.



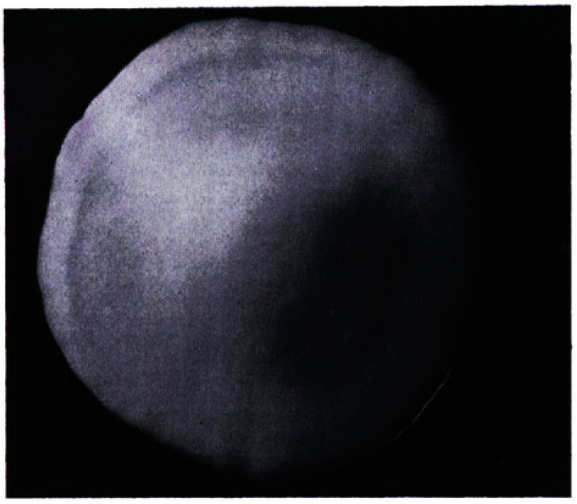
72. *B. bruxellensis*



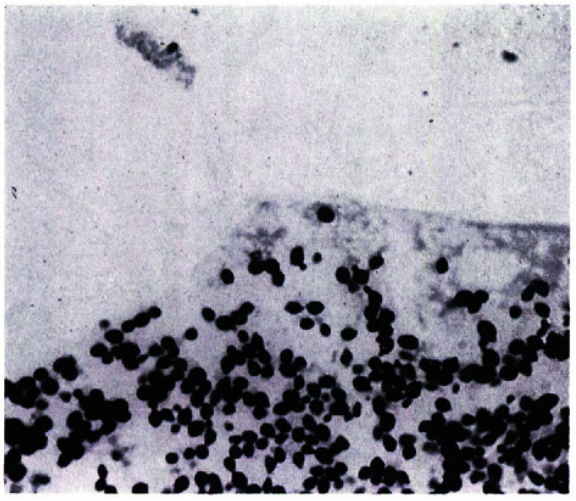
73. *B. claussenii*

Brettanomyces

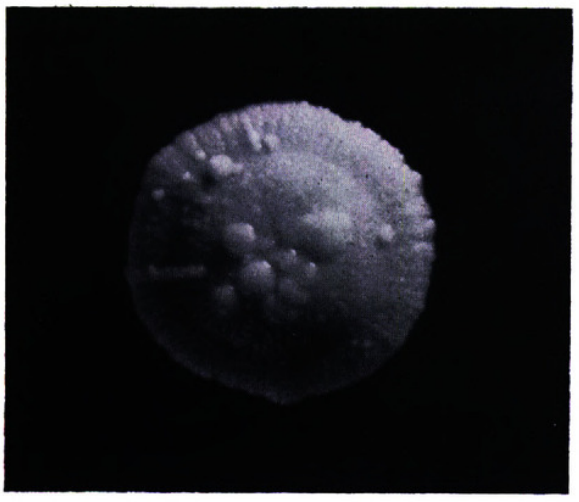
B. VERSATILIS ETCHELLS & BELL (Y–1016)



77. Giant colony grown on vegetable-juice agar; at 6 weeks. × 3.

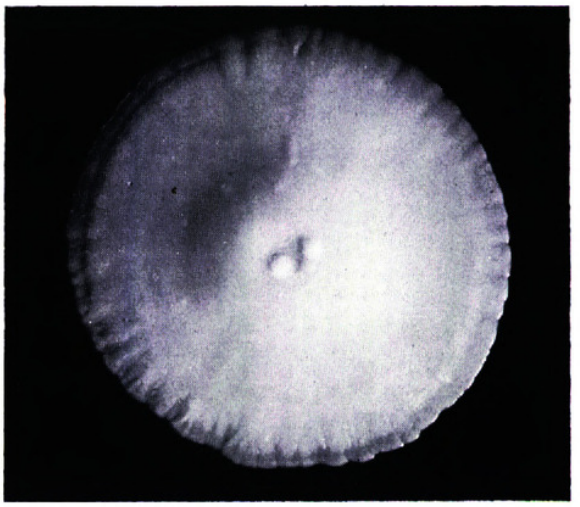


78. Cells from vegetable-juice agar at 1 month. Note pointedness of some cells. Gram stained, × 1500.

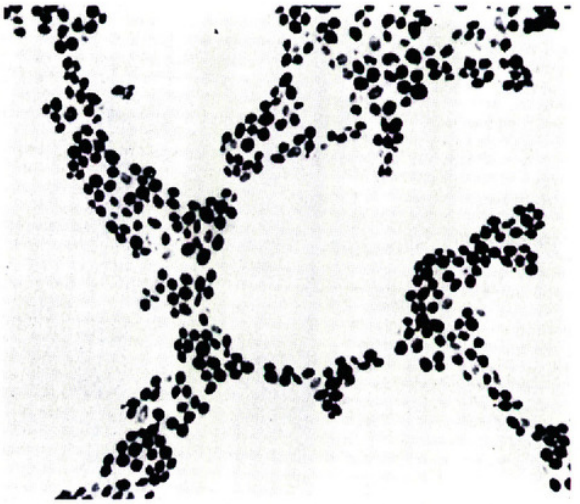


79. Giant colony grown on synthetic agar-A; at 6 weeks. × 4.

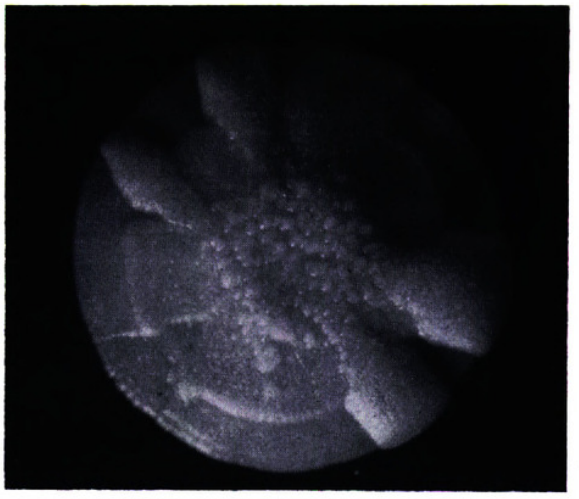
B. SPHAERICUS ETCHELLS & BELL (YS–606)



80. Giant colony grown on vegetable-juice agar; at 6 weeks. × 2.



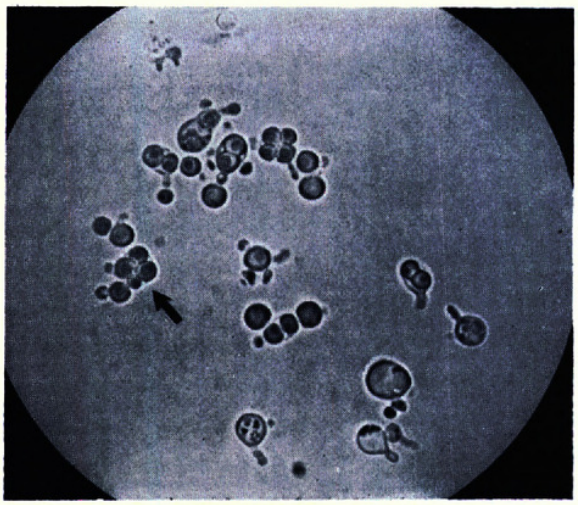
81. Cells from vegetable-juice agar at 1 month. Gram stained, × 1500.



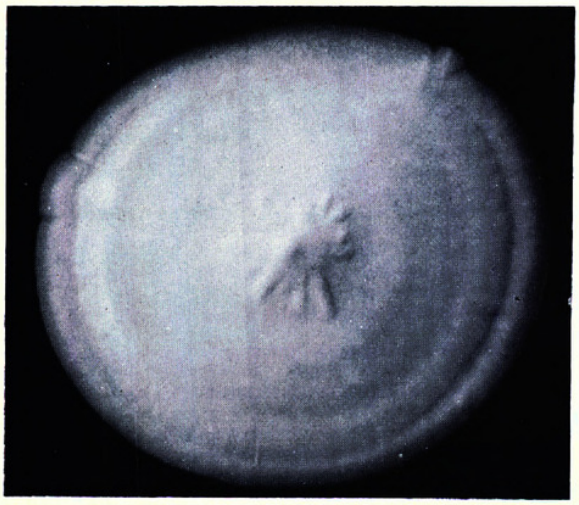
82. Giant colony grown on synthetic agar-A; at 6 weeks. × 4.

T. ROSEI LINDNER (RY-20)

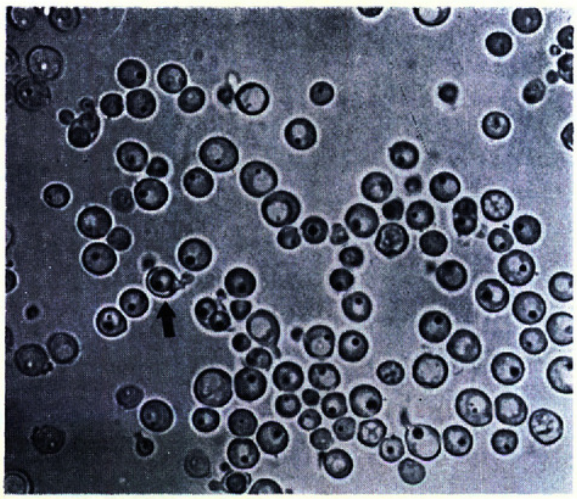
83. Giant colony grown on vegetable-juice agar; at 6 weeks. × 2.



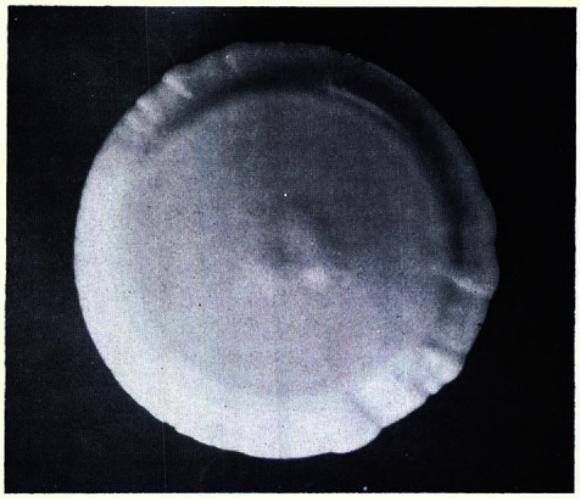
84. Sporulation on vegetable-juice agar at 1 month with 1, 2, and 4 (at arrow) round spores per ascus. Unstained, × 1500.



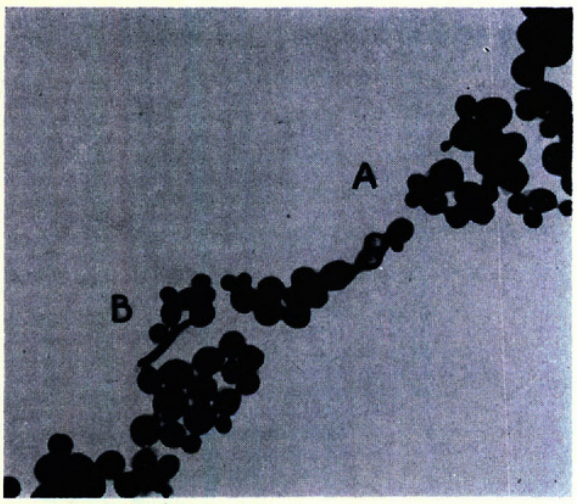
85. Giant colony grown on glucose agar; at 6 weeks. × 2.



86. Cells from a strain difficult to sporulate. Note single round spore at arrow. Grown on vegetable-juice agar; at 3 weeks. Unstained, × 1500.



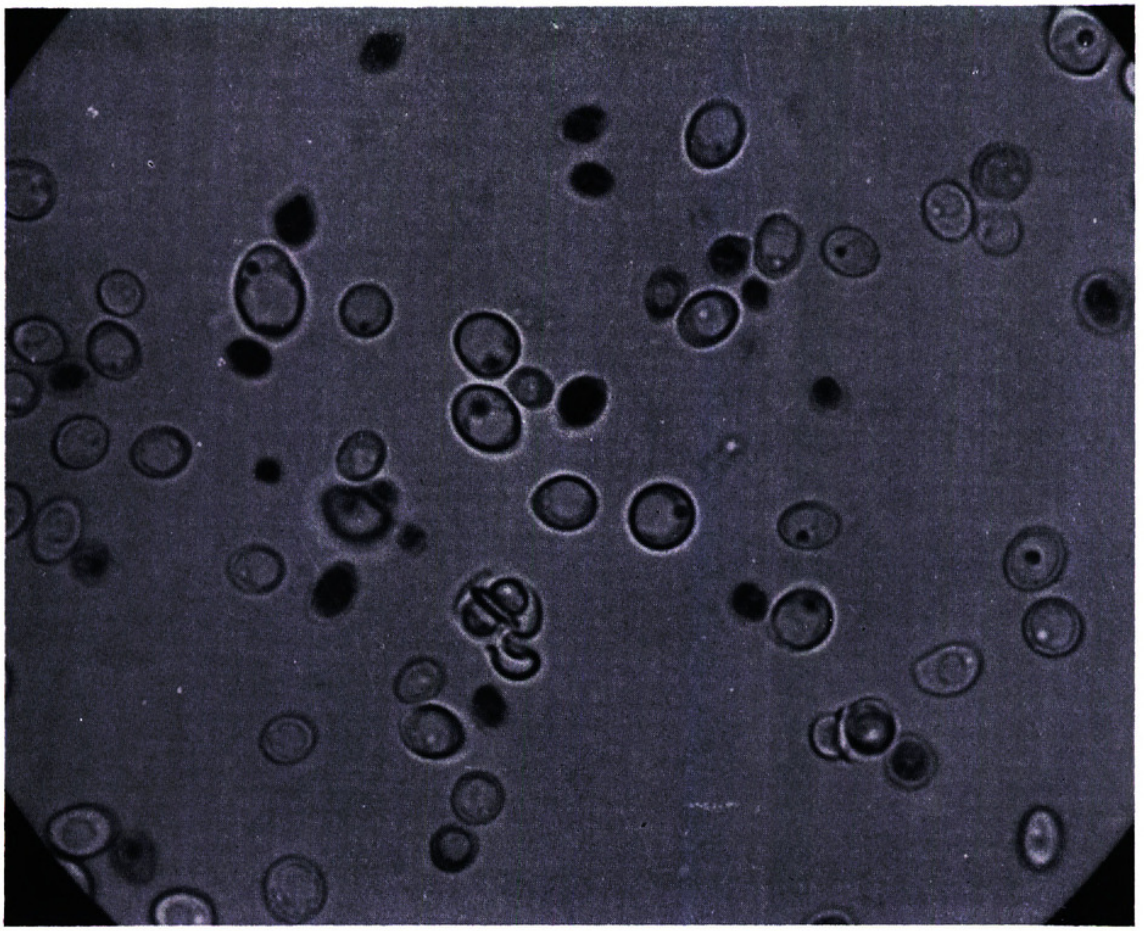
87. Giant colony grown on synthetic agar-A; at 6 weeks. × 3.



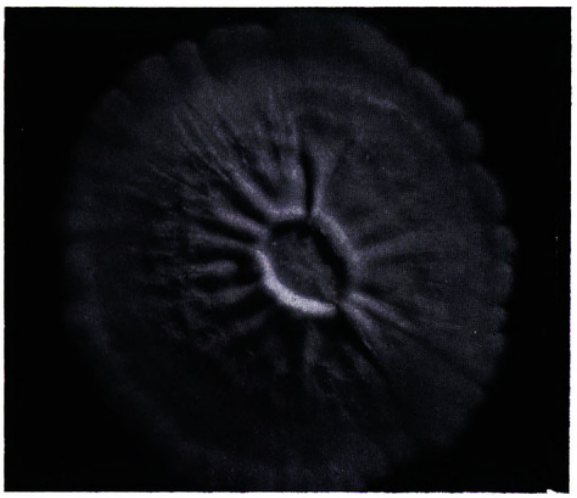
88. Cells from synthetic agar-A. Two unstained spores at *A*; long conjugation tube at *B*. Gram stained, × 1500.

Hansenula

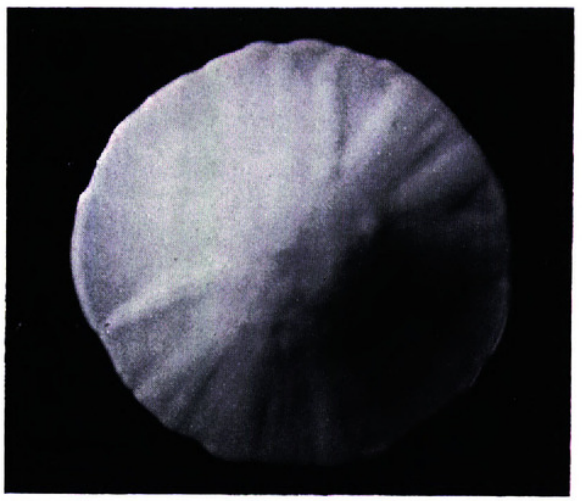
H. SUBPELLICULOSA BEDFORD (RY-135)



89. Sporulation on vegetable-juice agar at 1 month. Note two hat-shaped spores (brim to brim) emerging from ascus. Unstained, × 1500; enlarged, × 2.

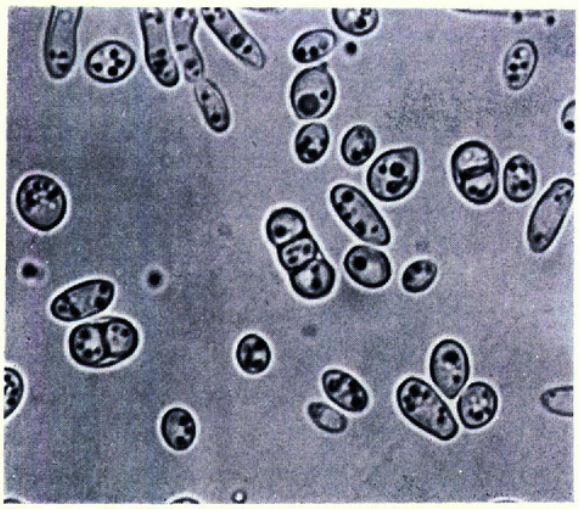


90. Giant colony grown on vegetable-juice agar; at 6 weeks. × 2½.

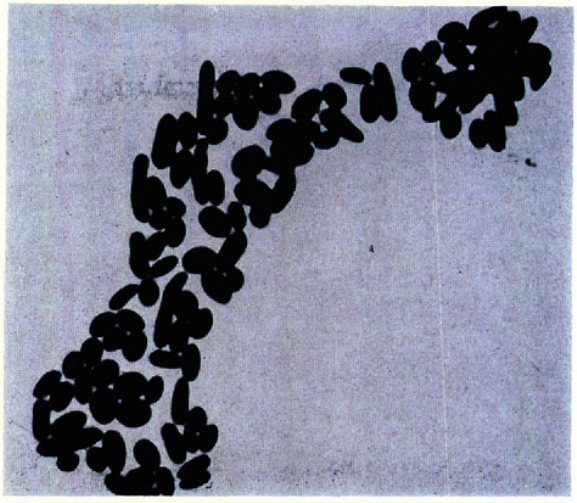


91. Giant colony grown on synthetic agar-A; at 6 weeks. × 3.

S. CEREVISIAE HANSEN (YD-15)

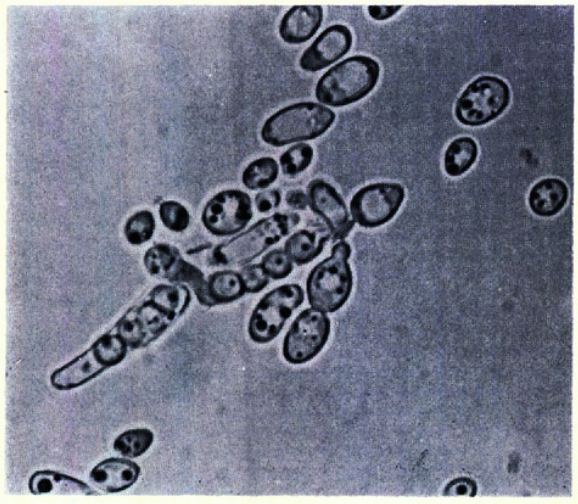


92. Two and 3 round to oval spores per ascus. From vegetable-juice agar at 2 months. Unstained, × 1500.

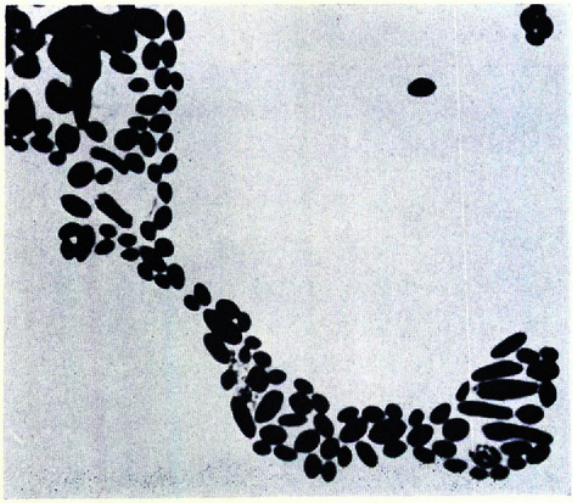


93. Cells from vegetable-juice agar; at 1 month. Gram stained, × 1500.

S. CEREVISIAE HANSEN (D-6)

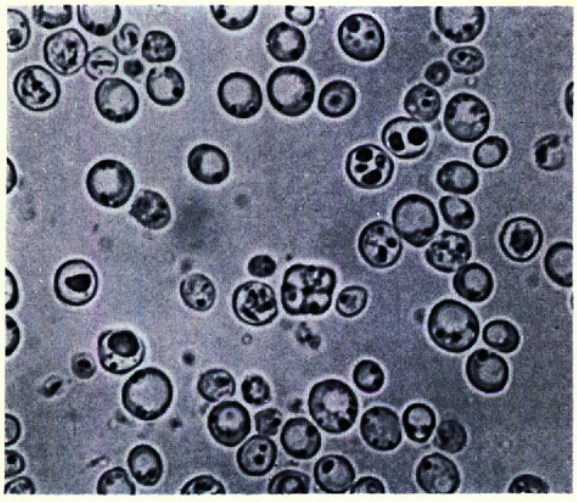


94. Asci with 4 round to oval spores each. From vegetable-juice agar at 1 month. Unstained, × 1500.

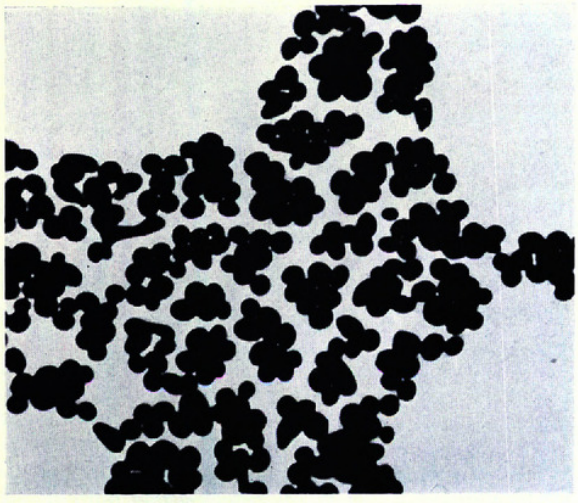


95. Cells from vegetable-juice agar at 1 month. Gram stained, × 1500.

S. GLOBOSUS OSTERW. (NY-114)



96. Typical round cells from vegetable-juice agar at 2 months. Four spores per ascus at lower center. Unstained, × 1500.

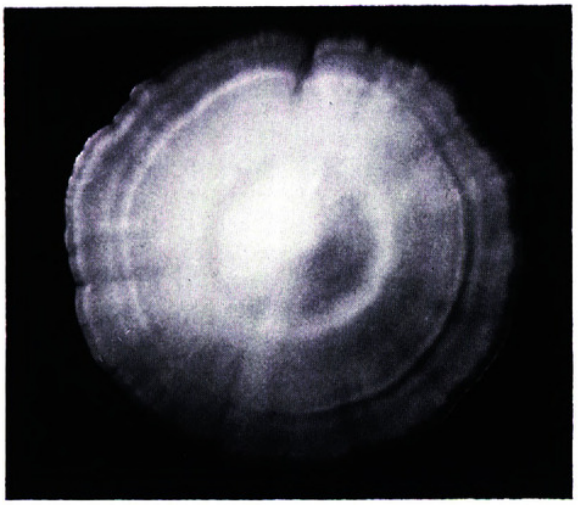


97. Young cells from synthetic agar-A at 7 days. Gram stained, × 1500.

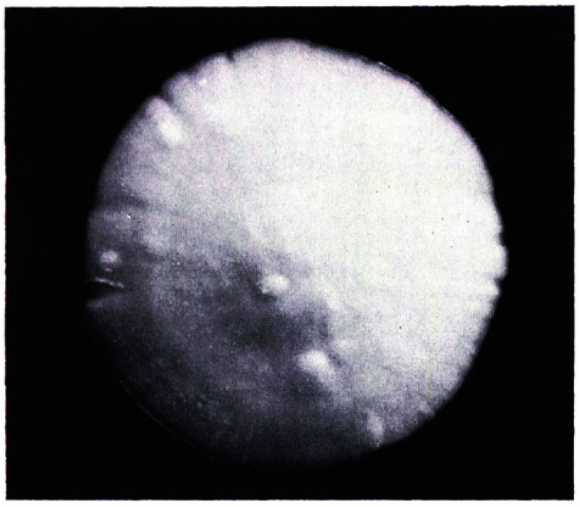
Zygosaccharomyces s.g.

Z. GLOBIFORMIS KR. & KB.

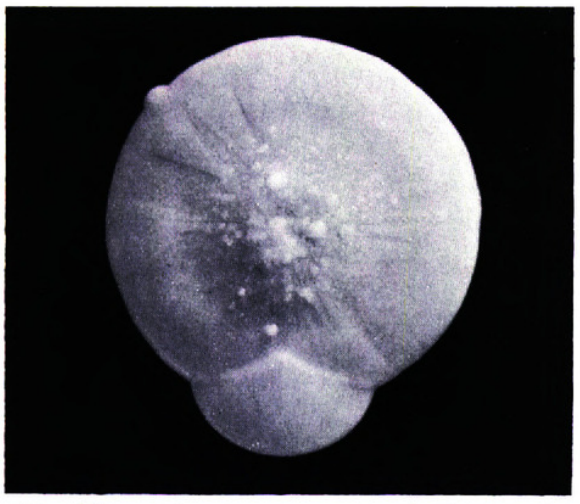
Y-742 from brined cucumbers



98. Giant colony grown on vegetable-juice agar; at 6 weeks. × 2½.

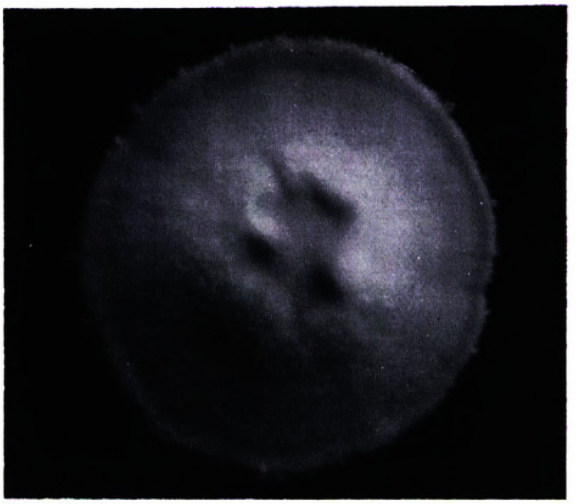


99. Giant colony grown on glucose agar; at 6 weeks. × 3½.

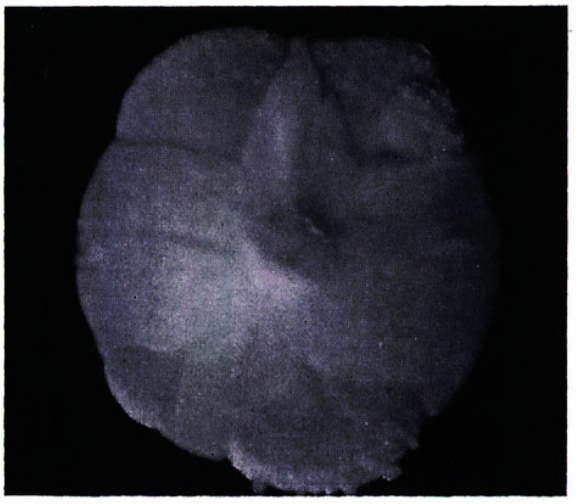


100. Giant colony grown on synthetic agar-A; at 6 weeks. × 3½.

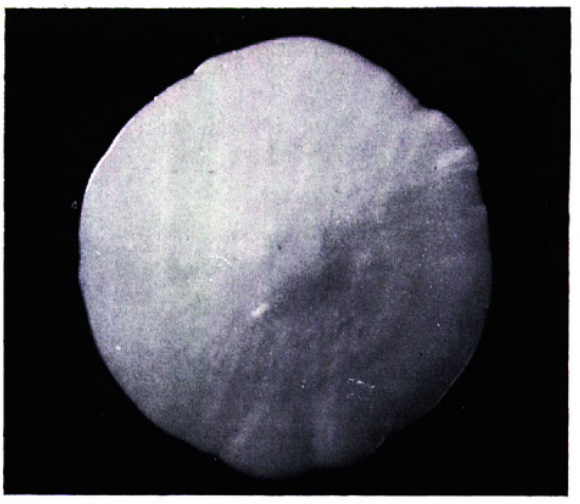
SPY-29 from spoiled sweet pickles



101. Giant colony grown on vegetable-juice agar; at 6 weeks. × 2½.



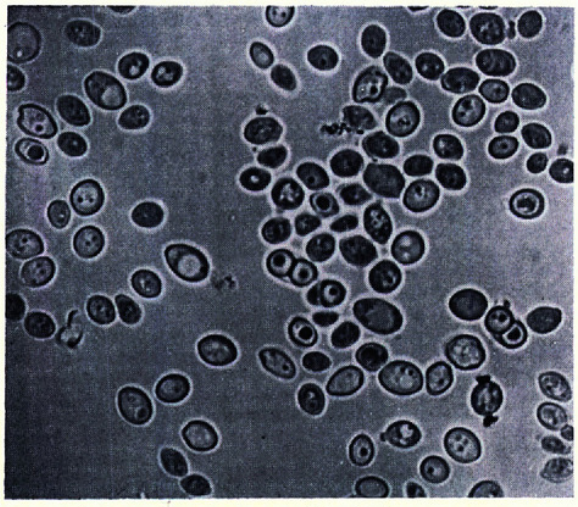
102. Giant colony grown on glucose agar; at 6 weeks. × 2½.



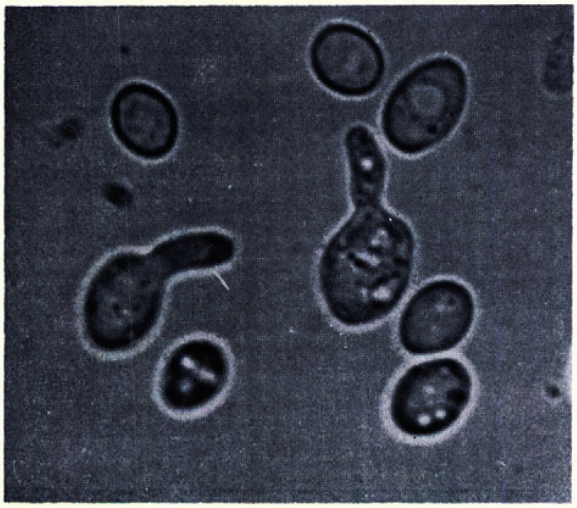
103. Giant colony grown on synthetic agar-A; at 6 weeks. × 3.

Z. GLOBIFORMIS (Cont.)

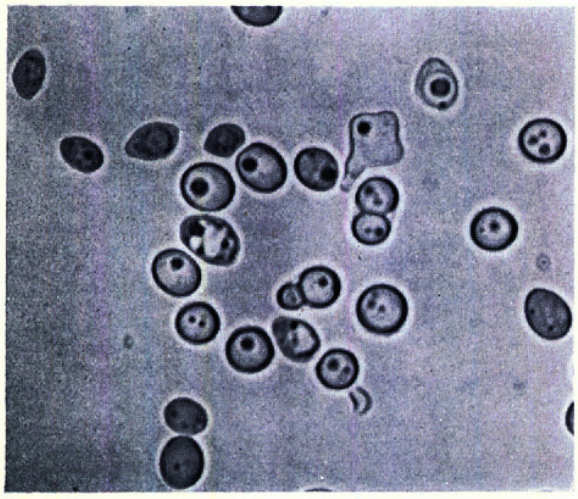
Y-742



104. Cells from vegetable-juice agar at 1 month with a few spores. Unstained, × 1500.

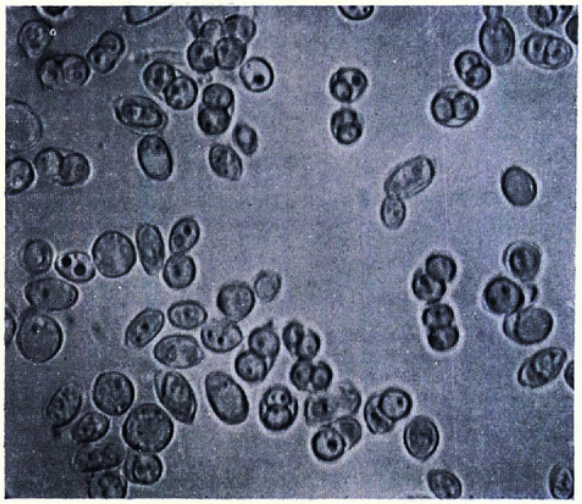


105. Cells with conjugation tubes from vegetable-juice agar at 1 month. Unstained, × 1500; enlarged, × 2.

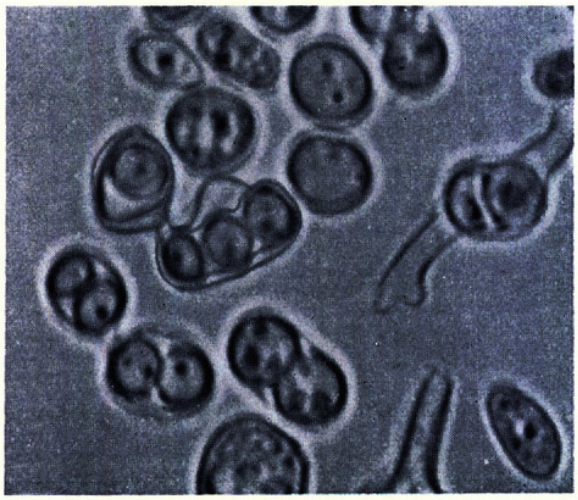


106. Free spores in center area, from vegetable-juice agar at 1 month. Unstained, × 1500; enlarged, × 2.

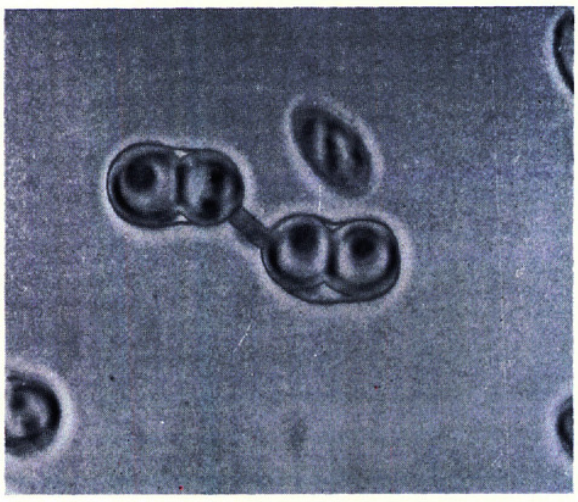
SPY-29



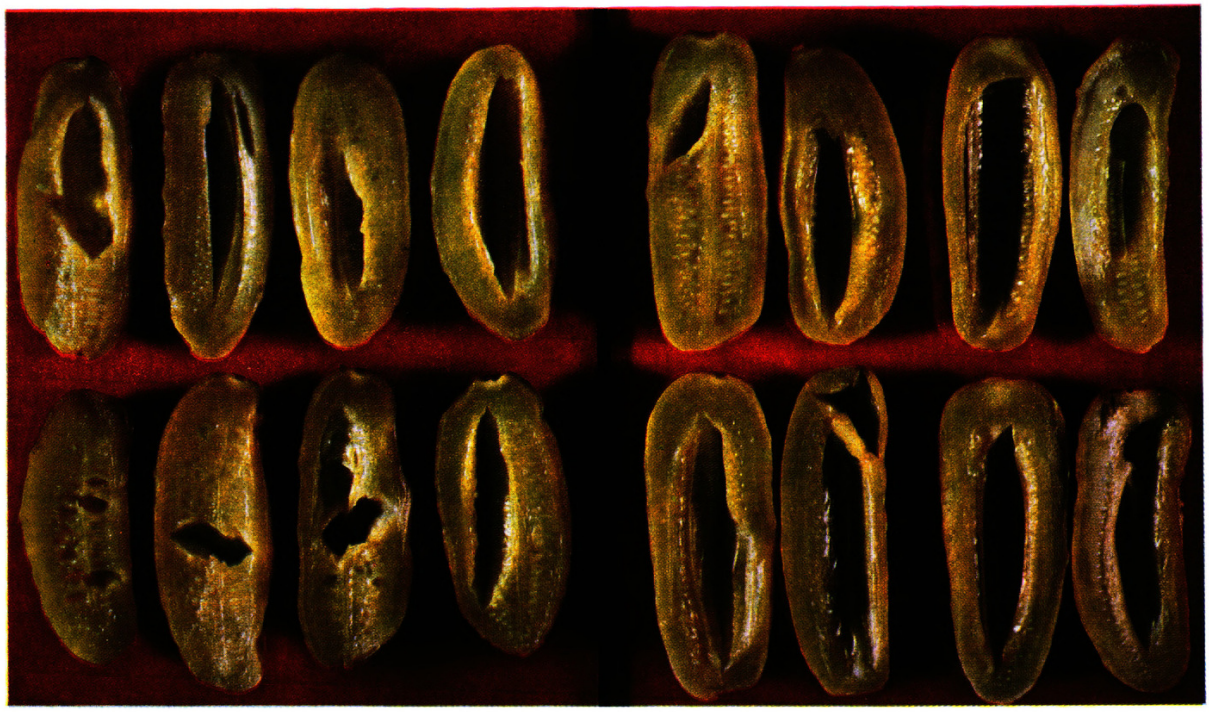
107. Sporulated culture from vegetable-juice agar at 1 month. Unstained, × 1500.



108. Asci with 4 spores. Center, 1 and 3 spores per side; lower left, 2 spores per side. Unstained, × 1500; enlarged, × 2.



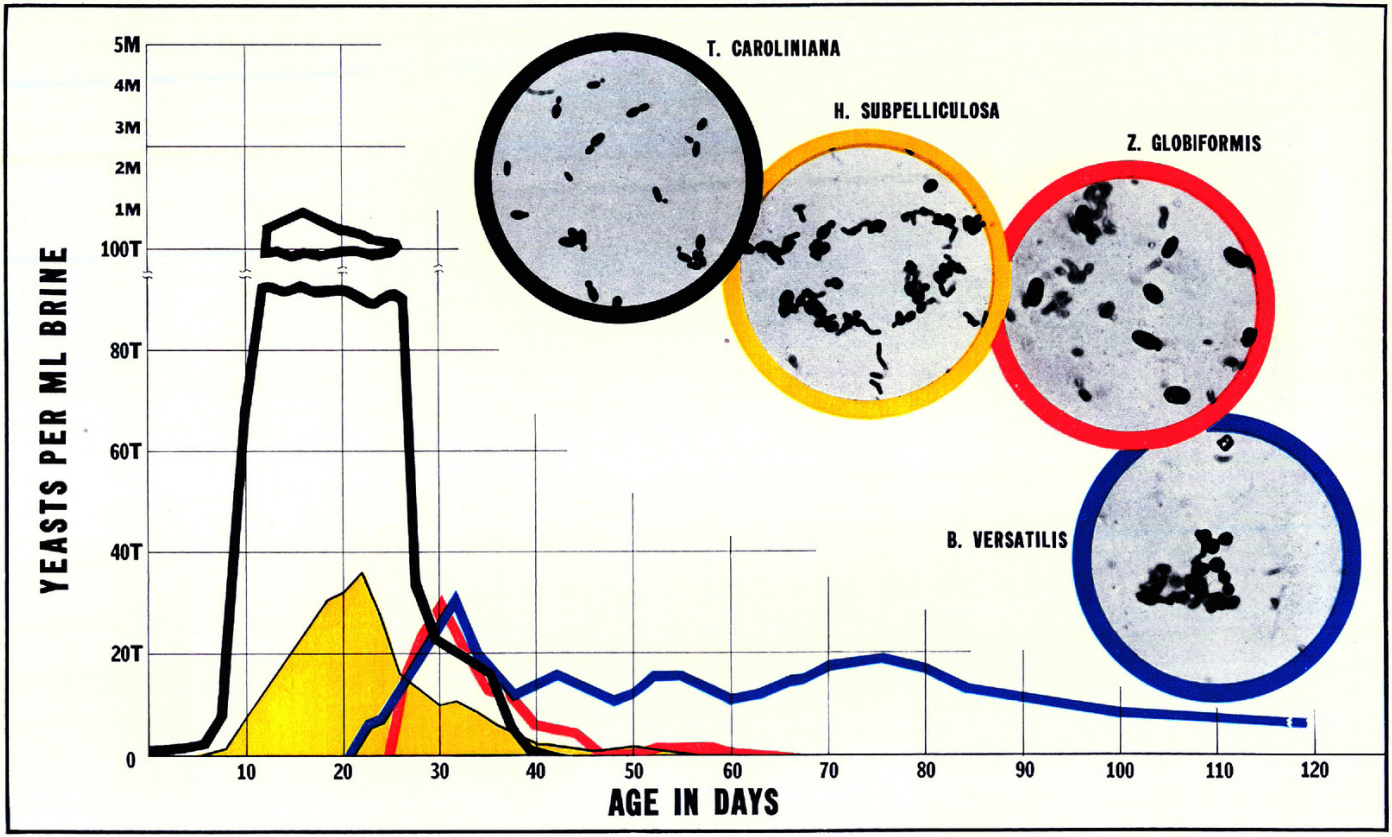
109. Ascus with two round spores per side. From vegetable-juice agar at 1 month. Unstained, × 1500; enlarged, × 2.



FROM NATURAL FERMENTATIONS FROM PURE CULTURE FERMENTATIONS

F. Examples of "bloater" formation by yeasts during the brine-fermentation of cucumbers. The four pairs of bloaters (at right) are typical of those produced by the 4 yeasts pictured below (p. 297).

110. BELOW. Yeast populations in commercial cucumber brines (T = thousands and M = millions) according to sequence of species (Cf. Etchells and Bell), (6). Inserts show 4 individual species as they occur naturally in brines during fermentation. Cells in brines Gram stained, × 1500.


T. CAROLINIANA
H. SUBPELLICULOSA
Z. GLOBIFORMIS
B. VERSATILIS
YEASTS PER ML BRINE
5M
4M
3M
2M
1M
100T
80T
60T
40T
20T
0
10
20
30
40
50
60
70
80
90
100
110
120
AGE IN DAYS

YEASTS FROM THE CUCUMBER PLANT

During the 1951 growing season, work was started to determine the numbers and species of yeasts associated with different parts of the cucumber plant *(Cucumis sativus)*. In the course of these studies, 966 yeast isolates were obtained from 37 sets of staminate and pistillate flowers, and five samples of small, immature fruit. These samples came from two important cucumber production areas in Eastern North Carolina. The details of the above study will be published elsewhere when the taxonomic work is complete; however, for our purpose here, certain remarks are in order.

More than one-half of the yeast cultures obtained during the study were asporogenic, non-fermentative, carotenoid-producing types placed in the genus *Rhodotorula*. Other yeast genera represented were: *Candida*, *Torulopsis*, *Debaryomyces*, *Torulaspora*, *Kloeckera*, *Saccharomyces s.s.*, and *Zygosaccharomyces s.g.* A breakdown of *Rhodotorula* isolates showed three major groups; red cultures similar to *R. glutinis*, yellow cultures similar to *R. flava*, and yellow cultures apparently not related to *R. flava*. In the minor red group were five rough strains with red-orange color; these produced rather well-developed mycelia. Several minor types were found among the yellow pigmented yeasts, including one that developed a latent black pigment.

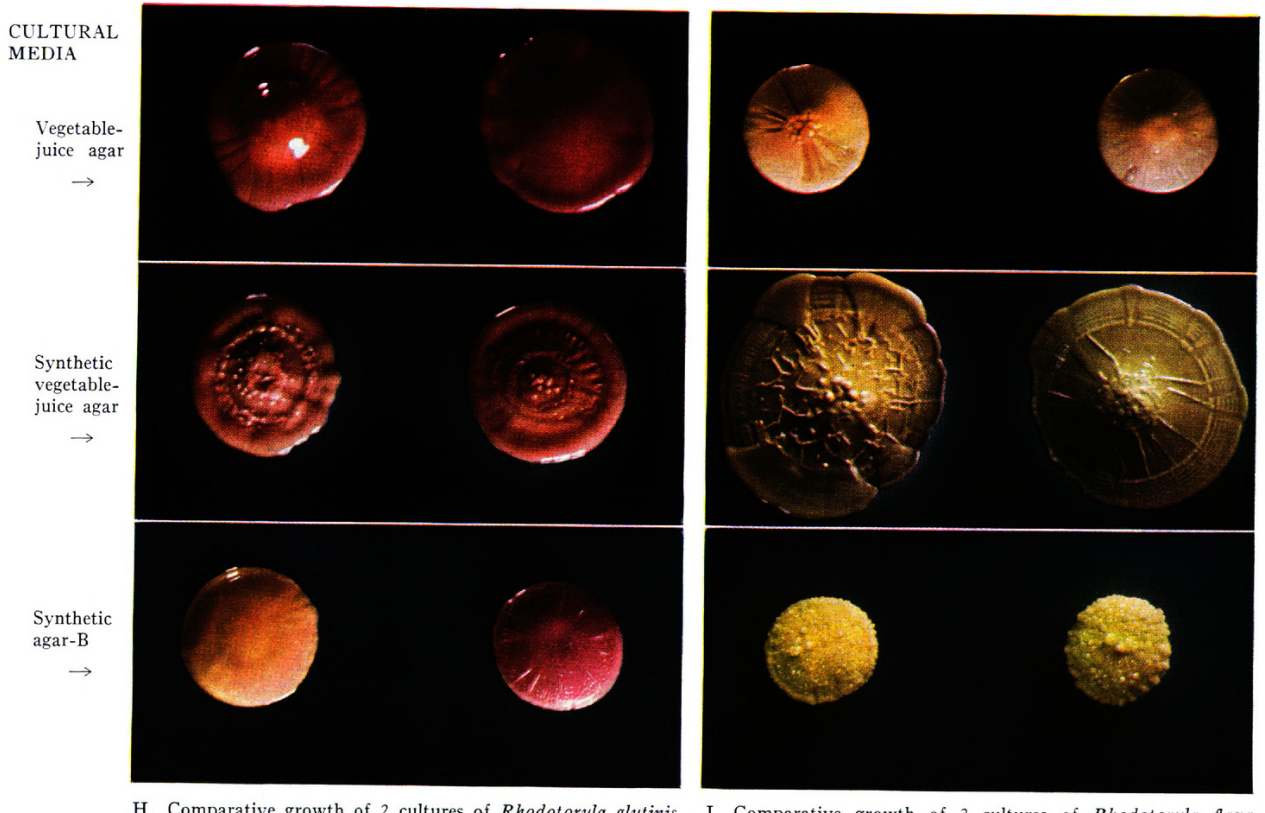
In order to meet the accepted requirements of the genus *Rhodotorula* the presence of carotenoid pigments must be demonstrated. Further, with a large collection of isolates, the use of culture media that will aid in visual screening of potential pigmented species is of importance, particularly for the yellow types. A large number of the latter yeasts would have been missed had they not first been cultured on SYNTHETIC AGAR-B. Finally, the need for improved cultural and chemical techniques to clearly demonstrate carotenoid production in pigmented species cannot be minimized. The use of strong acids and alkalis has been found inadequate for liberating the pigments from yeast cells grown on liquid or solid media of conventional type. However, excellent results were obtained for pigment extraction with acetone only, providing the yeasts were grown in SYNTHETIC BROTH-B for 72 hours on a rotary shaker. The pigments were then transferred to petroleum ether for characterization by chromatography and determinations of absorption spectra.

Based on current work, which will be reported in detail elsewhere, it seems clearly evident that carotenoid production covers a wider range of yeast types than previously suspected. In the pages to follow, absorption maxima for total carotenoid pigments in petroleum ether accompany the illustrations of giant colonies of certain of the *Rhodotorula* isolated. Also, chromatographs on magnesium oxide-supercel columns with petroleum ether were made on cell extracts from four yeast types. The spectral analyses of the carotenoid zones, in terms of visually observed absorption maxima in mμ are given below.

YEAST SY-85 (p. 300 LEFT), four pigments, Zone I, deep red, 485; II, red, 482 and 512; III, yellow, 460 and 488; IV, yellow (β-carotene), 450 and 475. YEAST SY-810 (p. 300 RIGHT), three pigments, Zone I, trace of pink; II, yellow orange, 450; III, yellow (β-carotene), 450 and 475. YEAST SY-875 (p. 301 RIGHT), four pigments, Zone I, deep red (sample lost); II, red, 435; III, yellow, 425 and 450; IV, yellow (β-carotene), 450 and 476. YEAST SY-836 (NOT SHOWN), one pigment, yellow (β-carotene), 450 and 474. β-carotene was identified as the pigment common to all four *Rhodotorula* species studied.

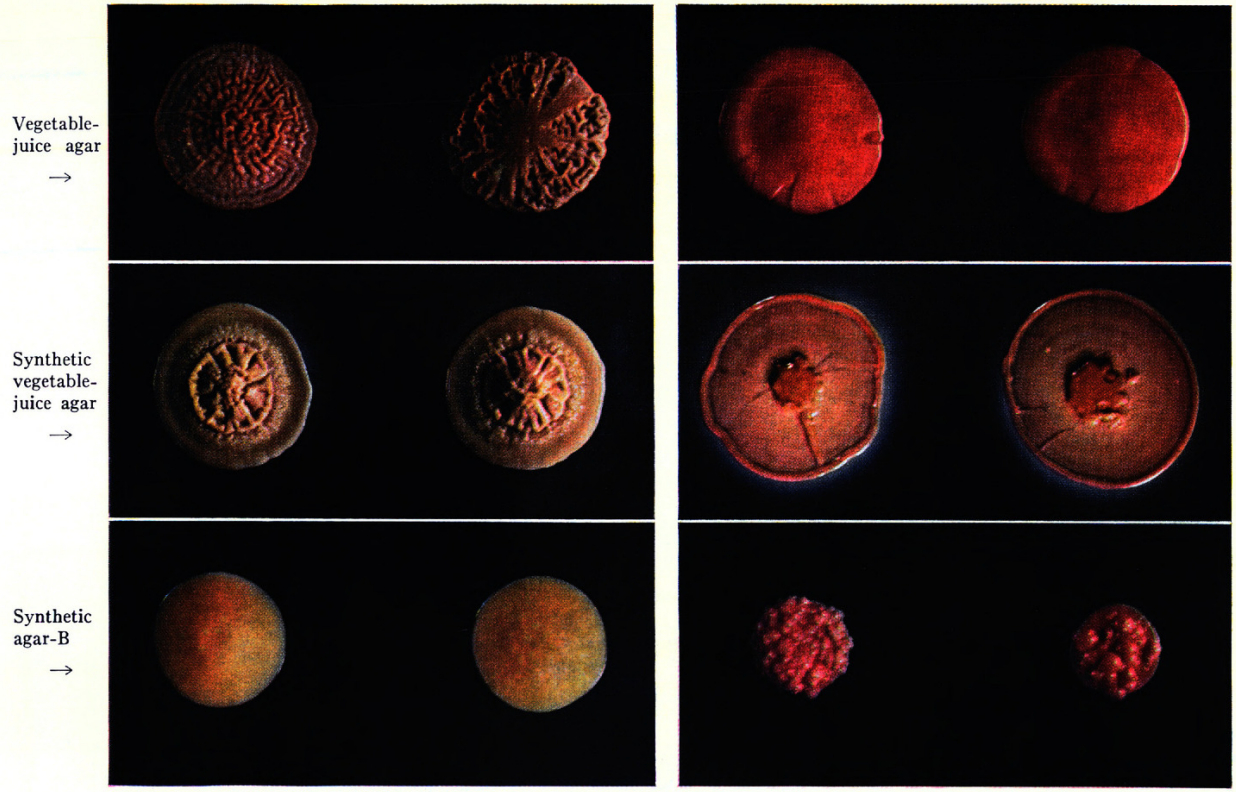
STAMINATE FLOWER AND BUDS | PISTILLATE FLOWER UNOPENED | PISTILLATE FLOWER OPENED | IMMATURE FRUIT WITH DRIED FLOWER

G. Flowers and immature fruit of the cucumber plant (*Cucumis sativus*). White spine variety; about actual size.



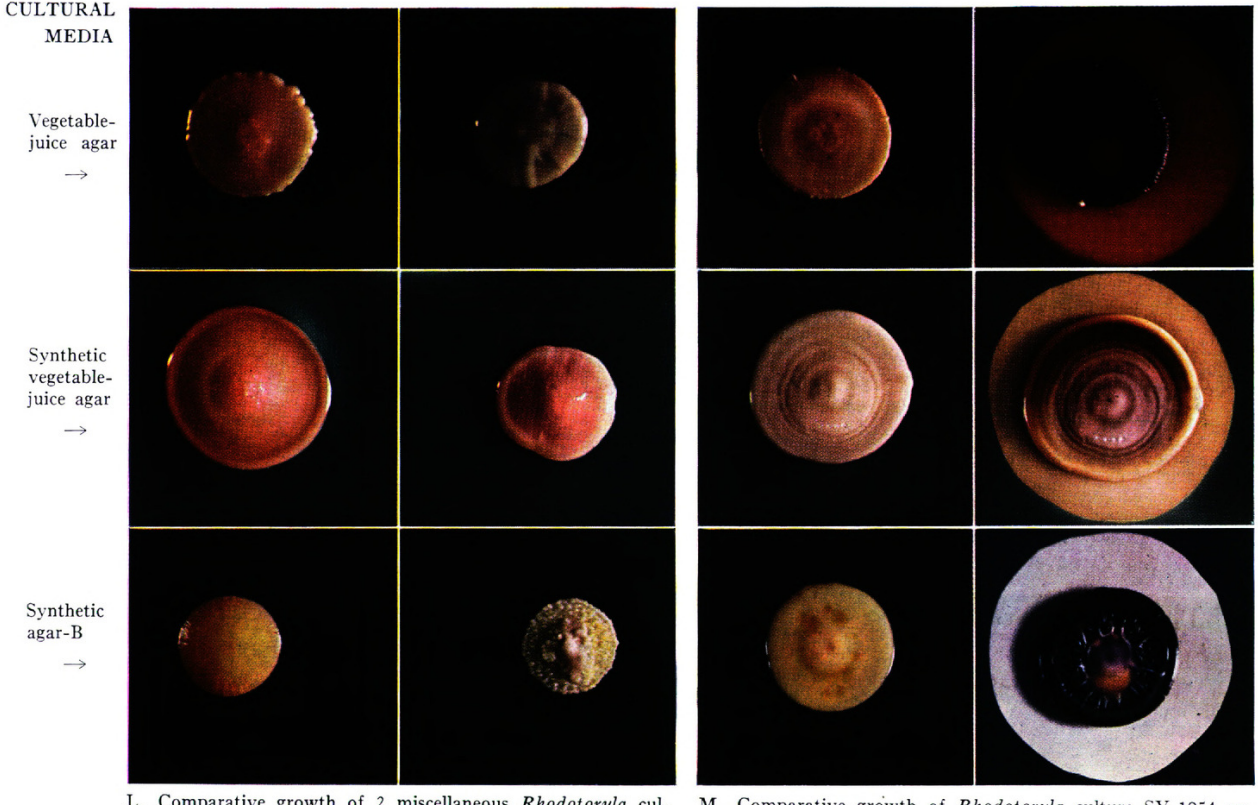
H. Comparative growth of 2 cultures of *Rhodotorula glutinis* group on 3 cultural media; 6 weeks' incubation at room temp.; × 1⅓. Absorption max. for culture SY-761 (left), 447 mμ; SY-85 (right), 480 mμ.

I. Comparative growth of 2 cultures of *Rhodotorula flava* group on 3 cultural media; 6 weeks' incubation at room temp.; × 1⅓. Absorption max. for both cultures, SY-873 (left) and SY-810 (right), 450 mμ.



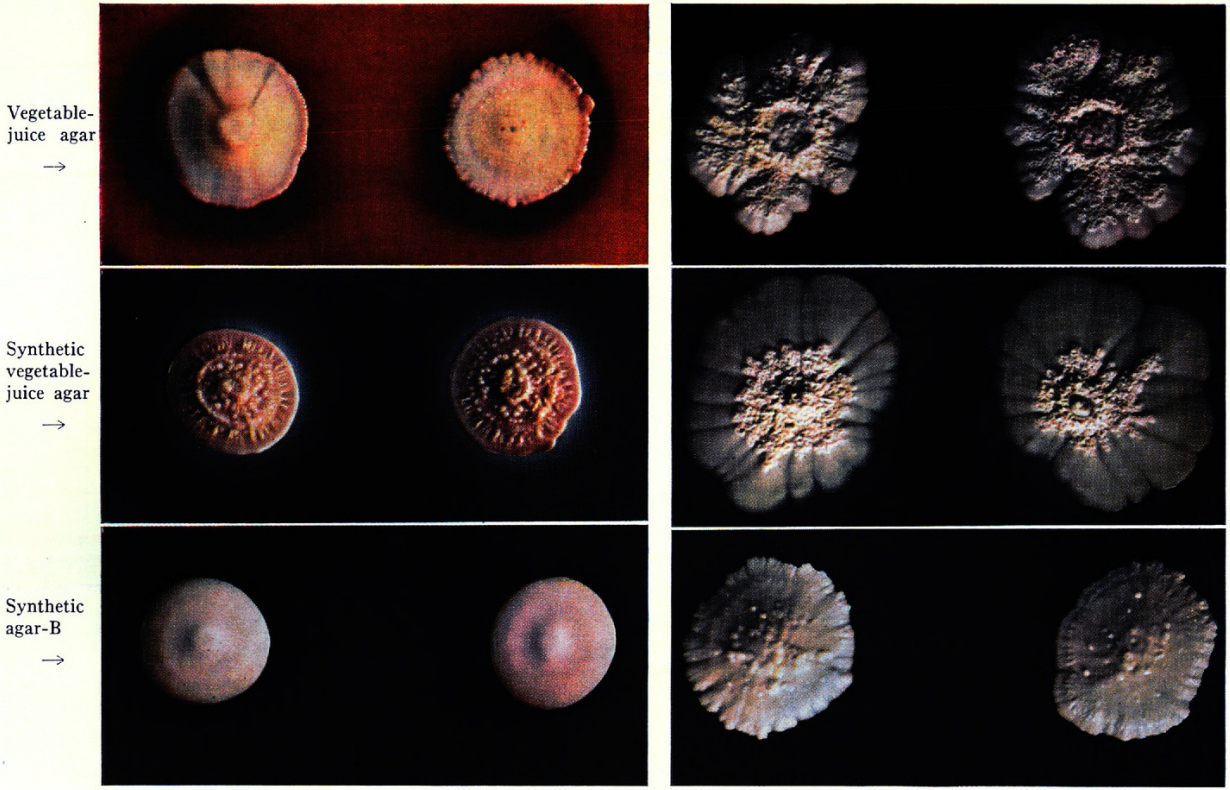
J. Comparative growth of 2 cultures of rough, yellow *Rhodotorula* species on 3 cultural media; 6 weeks' incubation at room temp.; × 1⅓. Absorption max. for both cultures, SY-629 (left) and SY-665 (right), 445 mμ.

K. Comparative growth of 2 cultures of rough, mycelia-producing *Rhodotorula* species on 3 cultural media; 6 weeks' incubation at room temp.; × 1⅓. Absorption max. for both cultures, SY-1070 (left) and SY-875 (right), 450 mμ.



L. Comparative growth of 2 miscellaneous *Rhodotorula* cultures on 3 cultural media; 6 weeks' incubation at room temp.; × 1⅓. Absorption max. for SY-364 (left), 450 mμ; SY-369 (right) not determined.

M. Comparative growth of *Rhodotorula* culture SY-1054 on 3 cultural media. *Left:* colonies grown 4 weeks at room temp. *Right:* the same colonies after an additional 3 weeks in the refrigerator (6°C.). Absorption max., 440 mμ.



N. Comparative growth of 2 pigmented cultures SY-161 (left) and SY-173 (right) of *Candida pulcherrima* on 3 cultural media; 6 weeks' incubation at room temp.; × 1⅓. Pigments soluble in the media; cells from both cultures negative for carotenoids.

O. Comparative growth of 2 cultures SY-188 (left) and SY-177 (right) of rough, fermentative *Candida* species on 3 cultural media; 6 weeks' incubation at room temp.; × 1⅓. Cells from both cultures negative for carotenoids.

ACKNOWLEDGMENTS

We wish to express sincere appreciation to the following Station personnel for generous assistance received: Dr. W. J. Peterson, Head, Department of Chemistry, and Dr. W. W. G. Smart, Jr., Department of Animal Industry, for their fine work on extraction and characterization of the carotenoid pigments; Messrs. N. S. Youngsteadt and Lane Palmer, Department of Publications, for their many hours devoted to planning, layout and art work; Mrs. Elsie Harper, Department of Visual Aids for special photographic printing; Dr. R. W. Cummings, Director of Research for the Station, and Professor M. E. Gardner, Head, Department of Horticulture, for their support and cooperation throughout this study. Notable contributions have also been made by Mr. A. L. Demain, University of California, who helped with isolation and identification studies during the summer of 1951; by Mr. L. P. Watson, Raleigh, N. C., who made the color pictures; and, by Mr. Robert Stevens, Lynchburg, Va., who was in charge of reproducing the natural color photographs. Our sincere thanks are given to Dr. Edgar V. Seeler, Editor of Farlowia, and the late Dr. W. Lawrence White, Director of the Farlow Library and Herbarium, Harvard University, for their advice and encouragement during the preparation of this article.

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