Y E A S T

A News Letter for Persons Interested in Yeast

November 1959 Volume VIII, Number 2

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The Editor takes pleasure in thanking all those who have contributed to this issue. Without this gratifying support the News Letter cannot fulfill its purpose. The Editors would like to invite others to send in contributions for future issues. It is planned to publish the next issue of the News Letter in May 1960. It would be appreciated if anyone would notify the Editor of additional people in our field who would like to receive the Yeast News Letter. Contributions to finance the 1960 issues are welcome. Many thanks to those who have recently contributed financially.

The Editors

I. C.B.S. Julianalaan 67 A-Delft, Holland. Communicated by Mrs. N. J. W. Kreger-van Rij.

The Yeast Division is now housed in a new building and the correct address is indicated above. Since our last report in the Yeast News Letter, the following new species (for which a description has been published) have been received by the C.B.S.

Candida lusitaniae van Uden et do Carmo-Sousa (N. van Uden, Portugaliae Acta Biologica (B), 6, 239, 1959).
Candida parapsilosis (Ashf.) Langeron et Guerra var. querci van Uden et do Carmo-Sousa (N. van Uden and L. do Carmo-Sousa, Portugaliae Acta Biologica (B), 6, 239, 1959).
Endomycopsis wickerhamii van der Walt (J. P. van der Walt, Antonie van Leeuwenhoek 25, 344, 1959).
Nadsonia slovaca Kockova et Svobdova (A. Kockova-Kratochvilova and Y. Svobdova-Polakova, J. gen. Microbiol., 20, 24, 1959).
Pichia robertsii van der Walt (J.P. van der Walt, Antonie van Leeuwenhoek 25, 337, 1959).
Rhodotorula glutinis (Fres.) Harrison var. daierensis Hasegawa et Banno (T. Hasegawa and I. Banno, J. Ferm. Technol. 36, 403, 1958).
Rhodotorula lactosa Hasegawa (T. Hasegawa, J. Gen. Applied Microbiol. 5, 30, 1959).

II. Universidade Do Recife, Instituto de Micologia, Av. Rosa e Silva, 347, Recife-Pernambuco - Brasil. Communicated by Prof. A. Chaves Batista.

The following papers have been prepared:

No. 242 - Endomycopsis clamitans n. sp. Batista & Coelho. This fungus does not ferment sugars but assimilates glucose, galactose, saccharose, maltose and potassium nitrate; it produces asci.

No. 241 - Endomycopsis interdigitalis n. sp. Batista & Coelho; this fungus ferments glucose, galactose and saccharose and assimilates glucose, galactose, saccharose, maltose and lactose; it does not assimilate potassium nitrate; it produces asci.

No. 239 - Debaryomyces artagaveytiae. n. sp. Batista, Silveira & Coelho. This fungus does not ferment sugars, assimilates glucose, galactose and saccharose; it does not assimilate potassium nitrate; it produces asci.

No. 238 - "Pesquisas microbiologicas sobre amostras de açucar demerara". Batista & de Lima. A research on raw-sugar with the identification of Torulopsis colliculosa (Hartman) Sacc., Torulopsis globosa (Olson & Hammer) Lodder & van Rij and Torulopsis dattila (Kluyver) Lodder, besides Leuconostoc mesenteroides.

No. 178 - "Endomycopsis dermatensis n. sp. isolado de lesões epidermicas do homen A. C. Batista & S.T.C. Campos & R. P. Coelho. This fungus ferments glucose and galactose; assimilates glucose, galactose and saccharose; it does not assimilate potassium nitrate; it produces asci.

Recent research: Yeasts from ammiotic liquid of women, are still being studied. (In 50 patients, Batista & V. Pereira, isolated 11 samples of yeasts identified

as Candida stellatoidea (Jones & Martin) Lang. & Guerra (1), Candida albicans (Robin) Berkh. (1), Candida parapsilosis (Ashf.) Lang. & Talice (4), Candida guilliermondii (Cast) Lang. & Guerra (3), Candida krusei (Cast.) Berkh. (1) and Torulopsis candida, (Saito) Lodder (1). These samples were taken through abdominal transparieto amniocentesis. These results will be published during December.

New Book. - "Monografia dos Fungos Micropeltaceae" by Dr. A. Chaves Batista, Publ. IMUR. 56, with 519 pages, illustr. has just been printed.

R. Pessôa Côelho left Zimology for a period of 6 months for health treatment.

Dr. Ulysses Correa has been appointed as Zymologist Chief in I.M.U.R.

Paulo Raulino Nascimento from Hospital Maracanaú Ceará, is here for a course on yeast fungi during one year.

R. Pêssôa Côelho, on October 7, 1959, held a public lecture on "Ascosporogenic yeasts found in feces".

III. Departamento de Microbiologia, Instituto Botanico, University of Lisbon, Portugal. Communicated by Dr. N. van Uden.

Since our last communication to the News Letter (volume VII, number 1) work on yeasts associated with man and other warm-blooded animals has continued and the following studies have been completed:

1. N. van Uden & M. Farinha, On the significance of temperature relations and vitamin deficiency patterns in the delimitation of yeast species, Portug. Acta Biol. (B), 6, 161, 1958.

Intraspecific variation of properties brought about by single-gene mutation, like dependence on external vitamins, though considerable in domesticated yeasts, may be insignificant in wild species, natural selection probably maintaining it low. Also temperature relations are fixed in at least several yeast species.

As an example of the possible importance of vitamin deficiency patterns and temperature relations in yeast taxonomy and ecology, results are given of a study of 8 strains labelled Candida zeylanoides (including type strains of the species and of two of its synonyms) and of the type strain of Candida zeylanoides var. norvegensis. The strains fall into two groups showing a perfect correlation between morphological and assimilative properties, vitamin deficiency patterns and temperature relations. With respect to the two latter properties C. zeylanoides (6 strains) is characterized by dependence on biotin and a maximum temperature of 30°-32°C., C. norvegensis nov. comb. (3 strains) by dependence on biotin, pyridoxin and thiamin and a maximum temperature of 41°-43°C.. On account of its maximum temperature C. zeylanoides is unable to grow in a warm-blooded host.

2. N. van Uden & L. do Carmo-Sousa, Further studies on the significance of temperature relations and vitamin deficiency patterns in yeast taxonomy. Portug. Acta Biol. (B), (accepted for publication).

In a total of 31 type strains and other strains, belonging to Candida parapsilosis and similar species, maximum temperatures, vitamin deficiency patterns and a number of other properties were studied. Perfect correlation between the two

former and the latter was observed in the strains studied. This constitutes further evidence that in wild yeast species the intra-specific variation of temperature relations and growth factor dependence is of a very low order and that the inclusion of these properties in taxonomic work is justified and useful. Emended descriptions of Candida parapsilosis;C. parapsilosis var. intermedia and C. obtusa nov. comb. are given. C. parapsilosis var. querci nov. var. and C. lusitaniae nov. sp. are described. All six strains of C. lusitaniae so far studied, came from the intestinal tract of warm-blooded animals.

3. N. van Uden, M. C. Braco Forte Jr. & L. do Carmo-Sousa, The occurrence of Cryptococcus neoformans in the equine intestinal tract, Bull. Off. Inter. Epizooties, 51, 82, 1959.

Cryptococcus neoformans was isolated from the caecal contents of a healthy horse belonging to a series of 252 equines sampled. A comparative study with regard to carbohydrate assimilation, vitamin requirements and maximum temperature for growth was made in 23 strains of C. neoformans with the following results: a. All strains utilize D-glucose, D-galactose, sucrose, maltose, D-xylose, D-mannitol, D-sorbitol and inositol. Most strains utilize raffinose, L-arabinose, ethanol, and soluble starch. Glycerol and inulin are weakly utilized or not at all. Lactose and nitrate are not assimilated. b. All strains are deficient for thiamin and grow well in the absence of other vitamins. c. Most strains have their maximum temperature between 39° and 40°C.; a few strains still show very weak growth at 40°C. but not above.

4. L. do Carmo-Sousa & N. van Uden, Estudo sobre a contaminação do iogurte com levedura. Bull. Pecuario, 36, 5, 1958.

From 103 samples of yoghurt produced by 4 factories in Lisbon, 70.8% showed contamination by yeasts of the genus Candida- C. pseudotropicalis, C. krusei and C. mycoderma. The number of cells per ml. reached 262,500,000 for C. pseudotropicalis and 57,000 for C. krusei. C. mycoderma was present in small numbers in 5 samples. C. pseudotropicalis was the most frequent and the most important contaminant. It causes an alcoholic fermentation in yoghurt, changing its odour, taste, consistency and texture. Yoghurt contaminated with large numbers of fermentative yeasts may be dangerous for debilitated people, originating or agravating intestinal dispepsia.

Our experiments showed that contaminations may be avoided or reduced to sporadic cases if: a)every utensil is well washed and afterwards submerged in water heated near 100°C during some minutes, preferably with detergents; b)ferment production is weekly renewed with pure cultures of Lactobacillus bulgaricus and Streptococcus thermophilus and is maintained in complete separation from the commercial production; c) the illegal addition of old yoghurt to the new-made lots is avoided.

It was also verified that: a) yeasts continue to multiply during storage even at ± 3°C.; b)C. pseudotropicalis resists 2 minutes at 65°C., C. krusei resists 1 minute at 75° C. and C. mycoderma resists 1 minute at 45°C.; casein particles may offer a certain protection; c)the more acid taste of contaminated yoghurt is not determined by a higher total acidity; d)for yeast counting modified Sabouraud medium is preferable to potato medium as the time of incubation is reduced to 48 hours; 3)smears are valueless when less than ± 5,000 cells per ml. are present; f)tests with brilliant green-lactose-peptone-bile medium for coliform organisms in yoghurt contaminated with lactose fermenting yeast may be falsely positive.

Besides the yeast species referred to above, Geotrichum candidum was found in 17 samples; no other fungi were observed.

5. H. Meleiro de Sousa & N. van Uden, On the mode of infection and reinfection in yeast vulvovaginitis, Am. J. Obst. & Gynec. (accepted for publication)

Evidence is brought forward suggesting that the intestinal tract is an important source of vaginal invasion by yeasts. Of 55 women with positive vaginal cultures of Candida albicans 42 (76%) harboured the same species in the faeces, whereas of a control group of 170 individuals only 43 (25%) had positive faecal cultures for C. albicans. A similar situation was found with respect to Torulopsis glabrata. After elimination of the yeasts from the vagina by local treatment faeces continued positive. Oral anti-yeast treatment is suggested to prevent reinfection by the anoperineal route.

Of 33 women with positive vaginal cultures for various yeast species 18 (54%) had also positive urine cultures; in each case yeasts isolated from vagina and urine belonged to the same species. In a control group of 91 women only 2 had yeasts in the urine. After local treatment of the vagina however, yeasts disappeared spontaneously and rapidly from the urine. It is concluded that the urethra is invaded by yeasts from an infected vagina, not vice versa, and that urine is normally not a source of vaginal invasion by yeasts and of infection and reinfection in yeast vulvovaginitis.

6. N. van Uden & L. do Carmo-Sousa, Some physiological properties of Geotrichum candidum, Mycologia (accepted for publication).

23 strains of various origins, including human and animal faeces as well as dairy products, morphologically suggestive of Geotrichum candidum showed a high degree of physiological uniformity:

a. All strains assimilate glucose, galactose, L-sorbose, D-xylose, ethyl alcohol, glycerol, D-mannitol, D-sorbitol.

b. No strain uses sucrose, maltose, lactose, cellobiose, trehalose, melibiose, raffinose, inulin, soluble starch, L-arabinose, L-rhamnose, i-erythritol, dulcitol, i-inositol, nitrate.

c. Fermentation of glucose and galactose is variable.

d. The maximum temperature for growth varies between 35° and 38° C., so not every strain is able to grow in a warm blooded host.

e. All strains split fat.

f. All but two strains are independent from external vitamins.

IV. Nagao Institute, 380 Mishuku, Setagaya-ku, Tokyo City, Japan. Communicated by Masami Soneda, Mycologist.

I am interested in Japanese wild yeasts and have accumulated many species. The first paper describing these results deals with 20 species of yeast, which were isolated from various kinds of animal dung collected at Zoological gardens and Mt. Fuji in Japan. Reference: Nagaoa-Mycological Journal of Nagao Institute, July 1959.

Following is summary of this report.

1) Twenty species of yeasts were isolated from various animal dung collected at Zoological Garden and the foot of Mt. Fuji.

2) Among them, Hansenula coprophila, Pichia minuscula, Candida fimetaria, Torulopsis fujisanensis and Kloeckera fluorescens are new to science. Candida brumptii and Torulopsis inconspicua are new addition to Japanese flora.

3) The yeasts inhabiting dung of carnivorous animals are present in relatively small numbers as compared with those of omnivorous and graminivorous animals so far as tested.

4) Strains of Rhodotorula were isolated only from dung of graminovorous animals, and strains of Kloeckera developed only from dung of wild animals.

5) Kloeckera fluorescens, isolated from wild animal dung, produces peculiar and distinct fluorescent matter which is yellowish under culture on malt agar. It shows bipolar budding on a broad base.
The greater part of the fluorescent matter was riboflavin, so far as tested.

Another publication is "Cultural and taxonomic studies on Prototheca" by K. Tubaki and M. Soneda. Nagaoa July 1959 page 25-34.

V. University of Miami, The Marine Laboratory, #1 Rickenbacker Causeway, Virginia Key, Miami 49, Florida. Communicated by Dr. S. P. Meyers and Miss Betty Ojeda.

This group is continuing their studies of the yeast population of littoral and deep sea environments. A paper dealing with investigations in Biscayne Bay, Florida, has been sent to the Journal of Bacteriology for publication. Recently, Dr. S. P. Meyers, with Dr. F. Roth Jr., Department of Microbiology, U. of Miami, as co-investigator, received a grant from the National Institute of Health for a three year study of the biology and metabolism of marine yeasts. Included in this work will be an evaluation of the biochemical niche that yeasts occupy in the sea. Other aspects include an evaluation of criteria acceptable for the differentiation of marine yeasts from their terrestrial counter-parts.

During our work we have collected various dark pigmented yeasts isolates that we have placed provisionally in the category "black yeasts". Since we recognize our lack of courage in this approach, we would like to hear from those workers interested in this group as well as any suggestions as to experimental approach to take these forms out of their taxonomic confusion.

We most welcome hearing from any workers who are interested in the marine yeast field as well as from those who are considering exploration along these lines. Isolates of marine yeasts, especially representatives of Rhodotorula, exhibiting any fastidious nutritional requirements, are always happily accepted.

VI. Laboratorio de Botanica-Facultad de Humanidades y Ciencias-Montevideo, Uruguay. Communicated by R. C. Artagaveytia-Allende.

"Yeasts isolated off the coast of Uruguay" is the name of an investigation published in Atti. Ist. Bot. Lab. Critt. Pavia, Italy.

Isolated were: Rh. mucilaginosa 4 strains, Sacch. cerevisiae 2, Rhod. flava 1, Cand. utilis 1, Cand. humicola 2, Cand. guilliermondii 1, Cand. intermedia 1, Cand. krusei 4, Cand. lipolytica 1, Cand. reukauffii 1, Geotrichum sp. 1, Tor. famata 2, from a total of 30 samples.

The findings were studied with relation to salinity and temperature of the water.

In this laboratory, Prof. Blanca Wermer de Garcia from the Dept. of Chemistry is actively studying yeasts. Also, Miss Dinorah Bracho from the Chem. Dept. of the Instituto de Investigacion Libre y Asesoramiento is working in wine microbiology as a result of a course (in this laboratory) on techniques of identification of yeasts.

VII. Dr. T. Hasegawa- Institute for Fermentation, Osaka, Japan, has published the following papers dealing with the genus Rhodotorula. (The first five papers are in Japanese, but have detailed English summaries; the last paper is in English).

Journal of Fermentation Technology, Vol. 34, No. 2. 1956. Studies on the Genus Rhodotorula Part I A Taxonomic Consideration on the Genus Rhodotorula.

Journal of Fermentation Technology, Vol. 36, No. 5, 1958. Studies on the Genus Rhodotorula (II) The Mycological Properties of the Rhodotorula Strains Preserved in Japan.

Journal of Fermentation Technology. Vol. 36, No. 10, 1958. Studies on the Genus Rhodotorula (III) On the Nitrate Utilization of Rhodotorula.

Journal of Fermentation Technology. Vol. 37, No. 5, 1959. Studies on the Genus Rhodotorula (IV) The Vitamin Requirement of Rhodotorula Species (1).

Journal of Fermentation Technology. Vol. 37, No. 5. 1959. Studies on the Genus Rhodotorula (V) The Vitamin Requirement of Rhodotorula Species (2).

The Lactose Assimilating Species in the Genus Rhodotorula. Reprinted from the Journal of General and Applied Microbiology, Vol. 5, Nos. 1-2, 1959.

VIII. Soil Bureau, Department of Scientific and Industrial Research, Eastern Hutt Road, Lower Hutt, New Zealand. Communicated by Dr. Margaret di Menna.

During the past year work on the distribution of yeasts in soil and on the leaves of pasture plants has continued. The seasonal pattern on leaves, of Rhodotorula spp. in summer and autumn and Cryptococcus laurentii and Torulopsis ingeniosa in winter and spring, has appeared with almost monotonous regularity. The only seasonal change that has been found in the soil yeast flora is a small increase in numbers during the warmer months of the year, but predominant species vary from soil type to soil type. It had been thought that only Cryptococcus spp. and non-fermenting Candida and Trichosporon spp. were common in New Zealand soils, but lately Hansenula spp. have been found to be characteristic of two soils, both ill-drained and with a pH in the region of 7.

An examination has been made of interactions between soil and leaf yeasts and the bacteria, streptomyces and fungi most common in these habitats in New Zealand. Streptomyces showed the greatest antibiotic activity against the yeasts tested, fungi the least. This work is being prepared for pulbication at the moment.

IX. Universidade de S. Paulo, Instituto Zimotecnico, Piracicaba, São Paulo, Brasil. Communicated by Rodolpho de Camargo.

Last month we presented for publication four papers related to yeasts and fermentation. A brief summary of each one follows:

1) Observations on the microflora of Cocoa fermentation in the State of São Paulo I. Quantitative analysis of the microorganisms occuring in the different stages of fermentation.

A numerical analysis is given of the average microflora of fermenting cocoa beans in the State of S. Paulo, Brasil. All the microorganisms involved in cocoa fermentation are discussed, including the actinomycetes, which are responsible for the unpleasant and undesirable musty odour, which is some times conferred to cocoa. The temperature and pH changes are also considered in this paper, as well as its relations to the sequence of microbial development throughout the fermentation.

2) Observations on the microflora of Cocoa fermentation in the State of São Paulo II. Yeasts occuring in the fermentation.

One-hundred-and-twenty-four yeast cultures were isolated from fermenting cocoa beans in the State of S. Paulo. Ninety-nine isolates showed to be asporogenous, and only 25 ascosporogenous. The yeast cultures obtained, in order of decreasing frequency, were: Geotrichum candidum, Candida krusei, Kloeckera apiculata, Torulopsis theobromae, Candida mycoderma, Pichia fermentans, Sacch. cerevisiae, Sacch. sp., Pichia membranaefaciens, Candida parapsilosis, Hansenula anomala, Trichosporon spp., Rhod. mucilaginosa and R. glutinis.

3) Observations on the microflora of Cocoa fermentation in the State of São Paulo III. Note on the occurrence of pectinolytic microorganisms.

A brief note which calls attention to the occurrence of Geotrichum candidum and Candida mycoderma in the fermentation of cocoa beans.

These three papers were written by R. de Camargo, Jorge Leme Junior and Alcides Martinelli Filho and presented at the Congress of the "Sociedade Brasileira para o Progresso da Ciencia" held in Salvador, Bahia, last July.

A fourth paper (authors: Alcides Martinelli Filho, Helcio Falanghe, R. Nelly Neder and R. de Camargo) was presented to the VII Congress of the Brazilian Society of Soil Science, under the title: - Comparative study of several culture media for the isolation of Yeasts and Fungi from soil.

A comparative study of several culture media was made in order to determine their efficiency in isolating fungi and yeast from soil. The following media were used: - Littman, Littman modified, PDA+4% oxgall, PDA+2.5% oxgall, PDA +Crystal Violet, PDA+Rose Bengal , PDA+oxytetracycline, and PDA: The littman medium showed to be the most useful in detecting greater number of fungi, but did not show the same efficiency in the isolation of yeasts.

The Instituto Zimotecnico has a cooperative project with the Instituto de Cacau da Bahia, and Rodolpho de Camargo, Alcides Martinelli Filho and Jorge Leme Junior are due to spend some time in Bahia in order to study the microbiological aspects of cocoa fermentation in that State, which is the largest cocoa producer in Brasil.

X. Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Saskatchewan. Communicated by Dr. W. A. Taber.

We have been studying the morphogenesis of the synnema of Graphium ulmi. This fungus grows first as a multipolar-budding, oxidative yeast, then as true mycelia. The yeast phase reduces selenite to the red elemental state but not without its growth being inhibited somewhat. The duration of the yeast phase is not altered by incorporation of cysteine or glutathione into the medium. Temperature does not influence the transformation of yeast to mycelial phase. A study of the morphogenesis of the yeast and mycelial phase is underway.

XI. Department of Food Science and Technology, University of California at Davis, California. Communicated by H. J. Phaff.

Publications

1. A new species of Schwanniomyces: Schwanniomyces alluvius by H. J. Phaff, M. W. Miller and Wm. Bridge Cooke. Antonie van Leeuwenhoek (2d issue 1960).

2. Nutritional requirements of Saccharomycopsis guttulata (Robin) Schionning by M. Shifrine and H. J. Phaff. Mycologia 51, 318, 1959.

Professor M. Yoneyama, Hiroshima University, Japan, is spending a year in my laboratory (October 1, 1959-October 1, 1960) to work in the area of yeast ecology and taxonomy. At present we are studying the yeasts associated with frass and the various stages of the life cycle of Scolytus bark beetles, which attack Abies and Pseudotsuga. Thus far our findings indicate that the principal yeasts found in the bark beetles Ips and Dentroctonus by Shifrine and Phaff (Mycologia 48, 41, 1956) - Pichia pini, Hansenula capsulata and Candida silvicola - are not present in Scolytus frass and larvae. There appears to be one principal species of yeast associated with Scolytus. This yeast is now under study. Dr. Miller and I are assembling data on yeasts isolated from a great variety of natural sources over a period of years and preparing it in the form of a short publication. We hope that such information may help in a better understanding of the habitat of yeasts, of which relatively few strains are available.

Dr. Shifrine and I are attempting to prove previously made hypothesis on the mechanism by which Saccharomycopsis guttulata becomes established in the gastro-intestinal tract of newly born rabbits.

Mr. Tanaka, graduate student in Microbiology, is working on certain aspects of the phenomenon of protein (enzyme) excretion by yeast.

XII. Southern Illinois University, Carbondale, Illinois. Communicated by Dr. Carl C. Lindegren.

Since the last publication of the Yeast News Letter, the following articles have been published or have been accepted for publication:

Desborough, Sharon and Lindegren, Gertrude. Chromosome mapping of linkage data from Saccharomyces by tetrad analysis. Genetica. Accepted for publication.

Lindegren, Carl C. Cancer and the respiratory grana. Nature 184, 397 (1959).

Lindegren, Carl C. Darwinism. Agrobiology (Russia) #5, 740-741 (Sept.-Oct., 1959).

McClary, Dan O., Nulty, W. L. and Miller, G. R. Effect of potassium versus sodium in the sporulation of Saccharomyces. J. Bacteriol. 78, 362-368 (1959).

Ogur, M., St. John, Ralph and Ogur, Sylvia. Direct experimental observation of cells in phenomic lag. Science 129, 1612-1613 (1959).

Ogur, Maurice, St. John, Ralph, Ogur, Sylvia and Mark, Abraham, M. Genetics 44, 483-496 (1959).

Pittman, David and Pedigo, Paul R. Photoreactivation studies on yeasts. I. Ultraviolet inactivation and photoreactivation of respiration-sufficient and respiration-deficient yeasts. Exptl. Cell Res. 17, 359-367 (1959).

Pittman, David, Ranganathan, B. and Wilson, F. Photoreactivation studies on yeasts. II. Photoreactivation of the ultraviolet damage producing respiratory deficiency in haploid and tetraploid yeasts. Exptl. Cell Res. 17, 368-377 (1959).

Carl C. Lindegren was elected to the office of President-elect of the Society of Illinois Bacteriologists at their October 30 meeting in Urbana, Illinois.

Mr. David Pittman of the Biological Research Laboratory has been invited to give a paper at the International Photobiological Congress to be held in Copenhagen on July 31-August 5, 1960.

XIII. The University of Wisconsin, College of Agriculture, Department of Bacteriology, Madison 6, Wisconsin. Communicated by Dr. H. O. Halvorson.

This past summer I was a visiting lecturer in the Dept. of Microbiology, University of Washington, Seattle and also had the opportunity of working on yeast genetics with Dr. D. Hawthorne and Dr. H. Roman in the new Dept. of Genetics.

We are continuing our interest in the biosynthesis and genetics of yeast β-glucosidase. Miss S. Winderman and Mrs. I. Winicov have discovered two types of β-glucosidase in S. fragilis and S. dobzhanskii. We are currently examining other mating systems for β-glucosidase and attempting to select for cellobiose positive S. cereviseae. We would appreciate examining any strains of the latter which may be available.

Dr. R. J. Young has recently joined our group. Together with Mr. H. Kihara they are examining the role of ribosomes in the synthesis of protein and induced enzymes.

Miss A. Herman and Mrs. F. Rupert have started their doctorate. They and Mr. John Gorman are examining a number of the related α-glucosidases. Several of the α-glucosidases and isomaltases are being purified, their induction inter-relationships examined and a comparison made of the proteins themselves.

IVX. Department of Bacteriology, McGill University, Montreal, Canada. Communicated by Dr. F. Blank.

THE CELL WALL POLYSACCHARIDES OF CANDIDA ALBICANS

We have investigated, together with Dr. C. T. Bishop, Dr. P. E. Gardner, Division of Applied Biology, National Research Council, Ottawa, the cell wall poly-

saccharides of C. albicans. Chitin was isolated (Scholl method) and identified by N-determination and X-ray diffraction analysis.

The powdered yeast cells were extracted with petroleum ether, the residue trypsinized, washed and freeze-dried. The residue was then refluxed with 3% NaOH for 15 hours. The solution was adjusted to pH 7.0 with $CH_3\ COO_H$ and dialyzed during 48 hours. The non-dialysables were precipitated with C_2H_5OH, redissolved in H_2O and precipitated with $C_2H_5\ OH$. The dried, N-free precipitate was shown by electrophoresis to contain two components which had mobilities of 4.35×10^{-5} and 9.34×10^{-5} cm^2, $volts^{-1}$, sec^{-1}. The two components were separated by a fractionation procedure and found to be a glucan and a mannan. The two polysaccharides were oxidized by periodate; consumption of the oxidant, production of formic acid, and cleavage products obtained after reduction and hydrolysis of the oxidized polysaccharides as well as methylation and subsequent hydrolysis showed that the glucan was a highly branched molecule with chains of β 1→6 linked glucose residues being joined together by β 1→ 3 glycosidic bonds. The glucans of C. albicans and S. cerevisiae are different. Although they have the same linkages the 1→6 linkages predominate in the C. albicans glucan whereas the 1→3 linkages predominate in the glucan isolated from S. cerevisiae.

In the same way, the mannan was shown to be an α 1→2 linked polysaccharide with branching occurring through C-6. The structure of the C. albicans mannan is the same as that of S. cerevisiae.

Publications: "Growth of Candida albicans on keratin as sole source of nitrogen" and "Growth of C. parapsilosis with keratin as sole source of nitrogen". Both authored by L. Kapica and F. Blank. Dermatologica 115, 81 (1957) and vol. 117, 433 (1958), respectively.

XV. Heriot-Watt College, Edinburgh, Scotland. Department of Applied Biochemistry, Microbiology Laboratory. Communicated by Dr. A. H. Rose.

A microbiological research laboratory has recently been opened in this department, and several research projects on yeast nutrition and metabolism are under way.

Dr. Rose has been studying excretion of free and combined forms of nicotinic acid by biotin-deficient Saccharomyces cerevisiae. Evidence has been obtained showing that the combined form of nicotinic acid excreted by the yeast is nicotinic acid adenine dinucleotide. A preliminary account of this work has been accepted for publication.

In collaboration with Mr. Fazal Ahmed, a student from Pakistan, a study of the nucleic acids in biotin-deficient Saccharomyces cerevisiae has been commenced. Preliminary results have shown that the pentose nucleic acid in this yeast is rather unstable compared with that found in yeast grown under conditions of optimal biotin.

Also in this laboratory, Mr. Per-Otto Hagen, from Norway, has begun studying the biochemistry of a psychrophilic species of Cryptococcus. Other research topics that are being studied in this laboratory by final year undergraduates include:

(a). Chemical composition of the capsular polysaccharide from the psychrophilic Cryptococcus.

(b). Aspects of the chemical composition of biotin-deficient Saccharomyces cerevisiae
(c). Hydrolysis of yeast mannan by micro-organisms.
(d). Fermentation of honey by pure cultures of yeasts.

Recent publications from this laboratory:

1. Rose, A. H. Beer. Scientific American, 1959, 200, 90.
2. Rose, A. H. Microbes in Industry, Science News, 1960, No. 1.
3. Rose, A. H. Excretion of nicotinic acid and nicotinic acid adenine dinucleotide by biotin-deficient yeast. Nature, in press.
4. Rose, A. H. Yeasts. Scientific American. in press.

XVI. Institut für Gärungsgewerbe, Mikrobiologische Abteilung, Seestr. 13, Berlin N 65 (West). Communicated by Dr. Siegfried Windisch.

During the last year the investigations were continued on vitamin requirements by beer yeasts. There appear to be certain relations between growth factor requirements and trisaccharide fermentation. (S. Windisch; On growth factor requirements and trisaccharide fermentation by beer yeasts. Brauerei, wiss. Beil. 12, 162, 1959).

Vegetative growth experiments were done with various materials, especially with beers. Results thus far obtained have shown that beer forms a secondary habitat, which is suitable for the growth of many species of yeasts and bacteria. Beer is biologically stabil and therefore does not spoil, when the various micro-organisms which occupy the habitat, are in equilibrium with each other. The yeasts can be determined so accurately that an analysis of their growth or multiplication can also be used as matter of plant or quality control. (S. Windisch: Growth studies with yeasts. Ber. deutschen Botan. Gesellschaft 72, 212, 1959).

Studies dealing with population genetics of yeast are being continued. The results thus far obtained have been published in a comprehensive paper. (H. Gutz and C. C. Emeis: Saccharomyces as a tool in studies on population genetics. Naturwissenschaften 14b, 457, 1959).

XVII. Cider and Fruit Juices Section, Research Station, Long Ashton, Bristol, England. Communicated by Dr. F. W. Beech.

The Cider and Fruit Juices Section is concerned with all aspects of these two products including academic research, pilot plant studies and acting as an official advisory centre to the two industries. Some advice is also given to wine importers and a few other miscellaneous industries.

The yeast work of the Section has been concerned with the isolation and identification of the flora of juices and ciders in our own cider house. Some of the results of this work have appeared in the following publications:

Beech, F. W. (1958) Yeast control in Cider Fermentations. Society of Chemical Industry Monograph No. 3, pp. 37-51.

Beech, F. W. (1959) The yeast flora of apple juices and ciders. J. Appl. Bact., 21. 257-266.

Beech, F. W. (1959) Some recent work on the microbiology of apple juices. Proceedings of a Symposium on Fruit Juice concentrates, Bristol 1958. Juris-Verlag, Zürich, Switzerland, pp 373-382.

There have also been papers on laboratory techniques e.g.

Beech, F. W. and Carr, J. G. (1955) A survey of inhibitory compounds for the separation of yeasts and bacteria in apple juices and ciders.

Beech, F. W. and Carr, J. G. (1958) Selective isolation of microorganisms. Chemical Products, 21, 285-287.

Beech, F. W. and Carr, J. G. (1959) Selective media for yeasts and bacteria.

Articles of a more general nature on cider-making have been published over the last ten years in the Annual Reports of the Research Station.

At present we are mainly concerned with the effects of sulphur dioxide on the microflora of juices and ciders and the microbiological problems concerned with the preparation and use of apple concentrate in the cider industry.

XVIII. Lake States Yeast & Chemical Division, 420 Lexington Ave., Suite 1634, New York 17, New York. Communicated by Mr. K. L. Cartwright.

It may interest the readers of "Yeast News Letter" that a cooperative study is underway among several nutritionists, biochemists, physicians and physiologists interested in various aspects of the role of d-alpha tocopherol in metabolism which will be aided by a special lot of Dried Torula Yeast U.S.P. made available by the Lake States Yeast & Chemical Division of St. Regis Paper Company, Rhinelander, Wisconsin.

The names of the investigators and their fields of interest may be obtained by writing to Mr. Cartwright. The analysis of the sample lot of yeast is given below.

One other development of industrial importance is the large scale manufacture of BAKON YEAST, a hickory wood smoked yeast used as a seasoning and nutritional supplement all in one. Descriptive information about this development may also be obtained.

Of prime interest to many nutritional workers is the clarification of the role of Factor 3 in animal nutrition and how it is distributed in yeasts. Yeasts grown in media containing enough selenium to provide 0.5 to several parts per million of Se per gram will have Factor 3 activity proportional to the level of selenium in the yeast. Factor 3 or selenium is known to be a partial replacement for d-alpha tocopherol under certain experimental conditions. To be useful as protein sources in E deficient experimental rations a yeast must contribute less than 0.05 ppm of selenium to the test diet. Since as much as 60% yeast is sometimes used in these diets, it cannot contain more than 0.08 ppm to be useful. Lake States Torula yeast fills this requirement. Average less than 0.05 ppm - probably less than 0.025 ppm. References: Nature (London) 182, 802, 1958; The Jour. of Nutrition 67, 433, 1959. A review on this subject may be obtained from Mr. Cartwright.

One final note of interest is the disparity between S. cerevisiae and C. utilis in their unsaturated fatty acid content. Dried Torula yeast U.S.P. (C. utilis) has between 2.5 - 4.5% linoleic acid while S. cerevisiae (Primary or Brewers) U.S.P. has less than 0.3%. Likewise, the natural yeast antioxidant is over six times as concentrated in Dried Torula yeast as in S. cerevisiae yeast.

DRIED TORULA YEAST U.S.P. FOR VITAMIN E RESEARCH

TYPE B - CONTROL NO. 58828R

Analyses		
Protein (N x 6.25)		54.6%
Moisture (105°C)		5.2%
Ash (1100°F-16 Hrs.)		7.64%
Total Fat (Acid Prehydrolysis)		7.06%
Unsaturated Fatty Acids		6.13% (86.8% of total fat)
Linoleic Acid		4.50% (63.7% of total fat)
Thiamin		187 mcgm/gm
Riboflavin		68
Niacin		671
Potassium		22.7 mg/gm (oven dry basis)
Phosphorus		14.9
Magnesium		1.9
Calcium		0.92
Zinc		0.18
Iron		0.12
Sodium		0.035
Silicon		0.025
Selenium	Less than	0.05 mcgm/gm (Neutron activation analysis)

AMINO ACID COMPOSITION
16% N (100% Protein) Basis

Figures are express in % of protein.

Lysine(1) 8.5; Methionine(1) 1.5; Cystine(2) 1.0; Tryptophan(1) 1.4; Phenylalanine(1) 5.1; Threonine(1) 5.1; Leucine(1) 8.0; Isoleucine(1) 6.4; Valine(1) 5.6; Arginine(3) 5.4; Histidine(3) 2.2; Tyrosine(4) 4.3; Glutamic Acid(4) 14.5; Glycine(4) 4.4; Alanine(4) 6.1; Aspartic Acid(4) 8.6; Proline(4) 0.5; Serine(4) 3.8.

(1) Essential for human adults

(2) Non-essential but can meet 15-20% of methionine requirements

(3) Essential for growing rats and possibly for growing infants

(4) Non-essential but utilized to meet total nitrogen requirements

XIX. Brief News Items and publications sent in by subscribers.

1. Dr. Austwick (Central Veterinary Laboratory, New Haw, Weybridge, England) visited Dr. Margarita Silva at the Department of Dermatology, College of Physicians and Surgeons, Columbia University after the Montreal meeting; also

Haskins Laboratories in New York. Discussions ranged from the problems in identifying the rarer yeast-like pathogens to the incidence and kinds of specialized enteric yeasts in interesting herbivora such as elephants, hippopotami, yaks, and giraffes. Studies are under way at Haskins Laboratories to test the versatility and dependability of isolation media for exacting "thermophilic" enteric yeasts. S. H. Hutner (Haskins Lab. New York).

2. I returned from Canada and the United States in mid-October after having had a most interesting visit. It is hardly possible to express my appreciation to the many friends from whom I received kindness and hospitality. Most of the Research Establishments which I visited were concerned with medical and veterinary mycology and hence all to some extend involved with yeast infections.

   There is only one item of interest from Weybridge. I.F. Keymer and I returned to the site of our isolation of Candida albicans from a lawn in July 1958 and on July 28th, 1959 were able to recover the fungus again from the same spot. This time no young partrides had been near the area and one must assume that it is a real habitat for C. albicans. We failed to isolate the yeast from grass in other parts of the same lawn and from the grass in adjacent fields. P. K. C. Austwick (Weybridge, England).

3. From October 1948 until June 1959 I did some mycological research work in Portuguese East Africa and special attention was given to the yeasts. Isolates were obtained from the intestinal tract of two groups of 100 natives, one on a very low animal protein diet, the other on a diet rich in animal proteins. Yeasts were also isolated from the intestinal tract of 119 wild animals, including hippopotami, wart hogs, bush pigs, baboons, and from water of lake Nyassa.

   Probably I shall participate in a conference on medical mycology, sponsored by the New York Academy of Sciences, to be held in New York in January 1960. I sincerely hope that it will be possible for me to meet yeast people and visit yeast laboratories on that occasion.

   We are planning to include some work on marine yeasts in our program and should appreciate to receive reprints and/or suggestions from people with any experience in this field. N. van Uden (University of Lisbon, Portugal).

4. Dr. Averill J. Wiley, Technical Director of the Sulphite Pulp Manufacturers' Research League, 1043 East South River Street, Appleton, Wisconsin writes - One personnel item may be of interest in that Dr. L. M. Whitmore, Jr., who has been Assistant Director on our League staff for the past 5 years and who has been much interested in yeast process and product research, advanced to employment with one of our member mills as Chief of Forest Chemicals Research at the West Coast Division of Scott Paper Co., Everett, Washington. This change in employment became effective last July.

5. Publications:
   Papers by Hiroshi Onishi (Noda Institute for Scientific Research, Noda City, Noda, Chiba Prefecture, Japan).
   "Studies on osmophilic yeasts" (in English)
   I Salt-tolerance and sugar tolerance of osmophilic soy yeasts. Bull. Agric. Chem. Soc. Japan 21, 137-142, 1957.

II Factors affecting growth of soy yeasts and others in high NaCl concentration. Ibid. 21, 143-150, 1957.

III Classification of osmophilic soy and miso yeasts. Ibid. 21, 151-156, 1957.

IV Change in permeability of cell membranes of the osmophilic yeasts and maintenance of their viability in saline medium. Ibid. 23, 332-339, 1959.

V (Continuation of paper II; effect of temperature) Ibid. 23, 351-358, 1959.

VI Glycerol production by salt tolerant yeasts in media with high concentrations of NaCl. Ibid. 23, 359-363, 1959.

The following two papers are by T. Takahashi and Y. Ikeda (Inst. Applied Microbiol. Univ. of Tokyo, Japan)

Bisexual mating reaction in Saccharomyces chevalieri; Genetics 44, 375-382, 1959.

Genetic analysis of α-methylglucoside fermentation in Saccharomyces. Zeitschr. f. Vererbungslehre 90, 66-73, 1959.

G. Terui, H. Okada, and Y. Oshima. "Studies on the correlation of alpha-glucosidase formation with genotypic composition in Saccharomyces (I) (in English). Technol. Reports Osaka Univ. 9, 237-259, 1959. Facult. Engineering, Osaka Univ.

Y. Oshima, On the fermentability of D-galactose, J. Ferm. Techn. 37, 418-425, 1959.

Y. Sasaki and T. Yoshida. A Taxonomic reconsideration on the alcohol fermenting yeasts preserved in Japan (Jananese with English key and summary). Mem. Facult. Agric. Hokkaido Univ. 3163-177, 1959, Sapporo, Japan.

From the Carlsberg Laboratory, Copenhagen, Denmark:

C. Roberts and A. T. Ganesan. The occurrence of multinucleate giant cells in yeasts. Antonie van Leeuwenhoek 25, 97-107, 1959.

A. T. Ganesan - The cytology of Saccharomyces.

A. T. Ganesan and C. Roberts. Observations on the nuclear cytology of Lipomyces lipofer. The last two occur in C. R. Trav. Labor. Carlsberg 31, 149-180, 1959.

From A. Sanchez-Marroquin (Escuela Nacional de Ciencias Quimicas. Ciudad Universitaria, Mexico 20, D. F.) "Accion de agentes antifungicos sobre levaduras del genero Candida" and "Effecto in vitro de combinaciones de agentes antifungicos. Revista Lationoamericana de Microbiol. 2, 89-98 and 99-110, 1959.

From N. Kawakami and coworkers (Dept. of fermentation Technol., Hiroshima Univ. Japan)

Electron microscopy of fungi II. Morphology of spores of yeasts by ultrathin sections - Bull. Fac. Engin. Hiroshima Univ. 5, 219-224, 1956.

VI "Sporulation and germination of Encomycopsis capsularis". Trans. Mycol. Soc. Japan. 9, 3-6, 1958.

VII. "Intracellular structure of Sporobolomyces salmonicolor". J. Ferm. Technol. 36, 393-396, 1958.

IX. "Intracellular structures of Rhodotorula glutinis and Nadsonia fulvescens and their relation to the physiological characters and taxonomic affinity. J. Ferm. Techn. 37, 125-132, 1959.

From Kirin Brewery Co. Research Laboratories, 17 Namaugi, Tsurumi, Yokohama, Japan,

we received their report of December 1958 (No. 1) 60 pages (in English). The issue contains articles on accumulation and changes of thiamine in yeast, isolation and estimation of glutathione and nucleic acids in yeast and an article on yeast flocculation.

From Caroline Hebb

C. Raut Hebb, J. Slebodnik, T. P. Singer, and P. Bernaud. On the nature of the block in the succinic oxidase system of anaerobically grown yeast. Arch. Biochem. Biophys. 83, 10-16, 1959.

From North Carolina Agric. Exp. Sta., Raleigh, N. C. "Influence of sorbic acid on the growth of certain species of bacteria, yeasts, and filamentous fungi. Jour. Bact. 77, 573-580, 1959.

Cornell Univ. Ithaca, New York. "Selection and isolation of auxotrophic yeast mutants with the aid of antibiotics. Jour. Bact. 77, 673-677, 1959. By A. G. Moat, N. Peters Jr., and A. M. Srb.

Letters to the Editor

Dear Sir:

The characterisation of Saccharomyces cerevisiae

The yeasts classified as S. cerevisiae and S. carlsbergensis are of industrial importance; and there is much biochemical literature on respiratory mechanisms in S. cerevisiae, usually as baker's yeast. The importance of this species makes it worthwhile to draw attention to the confusion about some of its biochemical characteristics, to review the facts briefly and to make some taxonomic recommendations.

Baker's yeast is almost always S. cerevisiae (13, 15, 19). Brewer's yeast is commonly so, though lager yeasts may be classified as S. carlsbergensis (6, 12, 18).

In agreement with Kudriavzev (11), work in this laboratory (unpublished) has shown that 8 authentic strains of S. cerevisiae were unable to grow on exogenous intermediates of the tricarboxylic acid cycle (citrate, fumarate, malate, succinate). This finding is also in agreement with observations on baker's and brewer's yeasts by a number of workers (e.g. 2, 4, 9, 10, 16, 17). An exception is the report by Krebs (8) of baker's yeast respiring α-ketoglutarate.

Kilkenny & Hinshelwood (7) reported that 5 strains of S. cerevisiae (designated A. B, C. D. E) grew on all the intermediates of the TCA cycle, except for strain C which did not use aconitic, citric, α-ketoglutaric and oxaloacetic acids. Probably none of these 5 were in fact strains of S. cerevisiae (Hinshelwood, 1957: personal communication). They were identified later at the CBS (Slooff, 1957: personal communication) as 3 strains of Hansenula anomala (A, B & D), one of Candida krusei (C), and one (E) of which the identity is doubtful. Strain E was first identified as Saccharomyces bayanus, subsequently as S. cerevisiae. Both these species have been reported to be incapable of growing on succinic, malic and citric acids (11). Moreover, in experiments in which the T-tube method (1) was used, this strain (CBS 2823) did not use these acids. Furthermore, NCYC 278, deposited as strain E (Brady, 1959: personal communication),

has been identified at the CBS as Candida guilliermondii (Kreger-van Rij, 1959: personal communication).

Although there are other reports of strains of S. cerevisiae utilising exogenous TCA cycle intermediates, they are not well substantiated either. For example, Beech (3) stated that a strain of this species ("S. ellipsoideus subsp. alpinus received by the CBS from Chodat in 1928") assimilated succinate and citrate; but Kreger-van Rij (1959: personal communication) could not confirm these findings on the same strain (CBS 1192).

It seems, therefore, that there may be some useful purpose served in characterising S. cerevisiae in such a way that all strains capable of metabolising any exogenous TCA cycle intermediates would be excluded from this species. A yeast which might well be excluded thus is Debaryomyces mandshuricus Naganishi, classified as S. cerevisiae by the CBS (5, 12). Kudriavzev (11) gave D. mandshuricus specific status, having obtained the strain he studied from Holland. He stated that it grew on succinic and malic acids, an observation which was confirmed for the CBS strain of this yeast by Kreger-van Rij (1959: personal communication). This suggestion that S. cerevisiae should be considered a group of yeasts unable to use exogenous intermediates of the TCA cycle accords with the view of Phaff et al. (14). They found that Zygosaccharomyces fermentati Naganishi, classified as S. cerevisiae var. ellipsoideus (5, 12), could use many carbon sources, including succinate, which unequivocal strains of S. cerevisiae could not. They changed the name Z. fermentati to Saccharomyces montanus. These observations of Phaff agree with those of Kudriavzev (11), though the latter kept the original name Z. fermentati.

There is evidence from unpublished work in this laboratory, and that of others (11, 14) that S. cerevisiae could be characterised usefully, also, by its inability to use exogenous polyols, e.g. mannitol, sorbitol, ribitol, erythritol and galactitol. The same characterization, with TCA cycle intermediates and with polyols, may well apply equally to yeasts usually classified as S. carlsbergensis, Saccharomyces uvarum and Saccharomyces logos. Each of these have been given specific status (12) almost entirely on cell-size, whereas the species "Saccharomyces ellipsoideus" was reduced to a variety on the same basis and its strains grouped in several species in the genus Saccharomyces that have widely different cell-sizes (Beech, 1957: personal communication).

It is urgent that yeast taxonomists should clarify this kind of information, at least for S. cerevisiae, the best known and most important yeast. If they are unable to agree internationally on such major characteristics for this species, they may find themselves in disrepute with biochemists and industrial workers to whom their work should be useful.

James A. Barnett
Low Temperature Research Station
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(1) Barnett & Ingram, 1955: J. Appl. Bact. 18, 131. (2) Barron et al., 1951: J. gen. Physiol. 34, 211. (3) Beech, 1957: The incidence and classification of cider yeasts, Bristol, Thesis. (4) Brandt, 1945: Acta physiol. scand. 10, suppl. xxx. (5) Centraalbureau voor Schimmelcultures. List of cultures 1957, Baarn. (6) Guilliermond, 1920: The yeasts, New York. (7) Kilkenny

and Hinshelwood, 1951: Proc. roy. Soc. B, 138, 375. (8) Krebs, 1935: Biochem. J. 29, 1620. (9) Krebs, 1949: 1st Int. Congr. Biochem. Abstr. p. 336. (10) Krebs, 1954: in Greenberg, Chemical pathways of metabolism, New York. (11) Kudriavzev, 1954: The systematics of yeasts, Moscow. (12) Lodder and Kreger-van Rij, 1952: The yeasts, Amsterdam. (13) Nickerson, 1957: in Roman, Yeasts, The Hague. (14) Phaff et al., 1956: Leeuwenhoek ned. Tijdschr. 22, 145. (15) Pyke, 1958: in Cook, The chemistry and biology of yeasts, New York. (16) Singer et al., 1957: Arch. Biochem. 69, 405. (17) Slonimski, 1953: Actualitos biochim. 17, 1. (18) Thorne, 1957: in Roman, Yeasts, The Hague. (19) White, 1954: Yeast technology, London.

Dear Sir:

Mr. Barnett has kindly shown me a copy of his very interesting contribution to the "Yeast News Letter . It prompts me to raise two points which I feel might be discussed very usefully in these columns or at the next International Congress of Microbiology in Montreal during August, 1962.

(1) Standardization of Test Methods.

In spite of the widespread use of Lodder and Kreger-van Rij's text book there does not seem to be universal agreement about the exact medium and method to be used for each of the yeast identification tests. One example is the growth test on different carbon sources, where four methods and at least two different media are in common use. The sensitivity of the methods varies considerably; sometimes a yeast will give no response with the auxanographic method but grow in liquid media.

Would it be possible to draw up an internationally agreed set of methods and media for yeasts such as is recommended by the Society of American Bacteriologists and published in their "Pure Culture Study of Bacteria"? Results could then be discussed without specifying their context. At present it is necessary to know in addition the method used and how positive, weak or negative results have been defined. The problem of "weak" reactions might be overcome if the results were expressed quantitatively as yield of yeast on a dry weight basis or checked by chromatographic examination of the growth media.

(2) Purity of Cultues.

Any proposed standardization of techniques should include provision for starting with a single cell isolate and preserving the first subcultures from it in a freeze-dried condition for future tests. This would minimize the risk of mutations in the culture; one suspects that cultures are sometimes discarded as being contaminated when in fact some mutation may have taken place.

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