Y E A S T

A News Letter for Persons Interested in Yeast

May 1963 Volume XII, Number 1

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Many thanks to those who have contributed to this issue by sending in news items and accounts of research projects. The next issue will be published in November 1963. A contribution of $0.50 from those who have not contributed for some time would be appreciated to finance future editions of the News Letter. Many thanks to those who have contributed recently.

The Editors

I. Centraalbureau voor Schimmelcultures, Yeast Division, Julianalaan 67A, Delft, Holland. Communicated by Mrs. N. J. W. Kreger-van Rij.

The following new species, for which a description was published, have been received by the C.B.S.

Candida guilliermondii (Cast.) Langeron et Guerra var. carpophila Phaff et Miller
H. J. Phaff & M. W. Miller, J. Insect Pathol., 3: 233, 1961.

Candida marina v. Uden et ZoBell
N. van Uden & C. E. ZoBell, Antonie van Leeuwenhoek, 28: 275, 1962.

Candida viswanathii Viswanathan et Randhawa.
R. Viswanathan & H. S. Randhawa, Science and Culture, 25: 86, 1959.
R. S. Sandhu and H. S. Randhawa, Mycopathologia, 18: 179, 1962.

Cryptococcus nigricans Rich et Stern
M. A. Rich & A. M. Stern, Mycopathologia, 9: 189, 1958.

Rhodotorula nitens Mackenzie et Auret
D. W. R. Mackenzie & B. J. Auret, J. gen. Microbiol., 31: 171, 1963.

Torulopsis maris v. Uden et ZoBell
N. van Uden & C. E. ZoBell, Antonie van Leeuwenhoek, 28: 275, 1962.

Torulopsis torresii v. Uden et ZoBell
N. van Uden & C. E. ZoBell, Antonie van Leeuwenhoek, 28: 275, 1962.

PUBLICATIONS:
N. J. W. Kreger-van Rij and F. Staib, The utilization of creatine and creatinine by some Debaryomyces species, Arch. Mikrobiol., 45: 115, 1963.

II. Division of Dermatology, University of California, School of Medicine, Los Angeles 24. Communicated by Dr. H. J. Shadomy.

In an effort to aid in the classification of the genus Pityrosporum, the 25 strains in our culture collection have recently been tested for possible ascospore production. This work was initiated primarily because of the spore-mother cell sac-like cells seen in preparations of a number of our organisms. The "ascus"-like appearance is most striking when viewed with the phase microscope. No large number of these cells were seen in a single preparation, but they were found frequently enough to encourage the study.

It was decided to grow the cultures on various ascospore stimulating media both along and crossed with each of the other cultures in the collection. This project was initiated on the gypseum blocks described in Henrici's Molds, Yeasts and the Actinomycetes, then carried on to the following media: Gorodkowa's medium and malt extract agar (from the above reference), and the McKelvey carrot infusion sporulation agar and Henrici's vegetable juice sporulation agar described in Littman and Zimmerman's Cryptococcus.

At appropriate times up to 8 weeks post inoculation, smears were made on numbered slides in a random fashion to assure impartial scanning, and stained by the Mittwer and Bartholomew modification of the Schaeffer and Fulton spore stain. Saccharomyces cerevisiae smears were carried through the staining procedure with each batch of the Pityrosporum as a control on the spore staining activity.

Results were uniformly negative throughout the study. That is, in every case spores were found on the Saccharomyces smear slides and none was ever seen on any of the Pityrosporum slides.

Due to the great number of samples run, including special studies made by heating of the slides to steaming with malachite green covering smears made both with and without treatment by acetone, indicate to us that there was no ascospore production and that the genus Pityrosporum is asporogenous.

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

1. Mr. Ismet Taysi, after having spent a year and a half in this laboratory working on a marine yeast project, returned to Turkey. Miss Helen Buckley from New York extended her stay for another year and is now working on the taxonomy of the "black yeasts". Dr. Sukeyuki Kawakita from Juntendo University, Tokyo, joined our group for a year's stay. He is working on the intestinal yeast flora of marine birds. Dr. Lidia do Carmo Sousa returned last fall from the United States and is busy with a revision of the genus Trichosporon; she is now spending a few months at the C.B.S. in Delft.

2. The main job of the moment is concerned with the taxonomic revision of five yeast genera that will constitute this laboratory's contribution to the second edition of Lodder and Kreger-van Rij's "The Yeasts". The work has been distributed as follows:

Candida (N. van Uden and H. Buckley)
Torulopsis (N. van Uden and M. Vidal Leiria)
Trichosporon (L. do Carmo Sousa and N. van Uden)
Nematospora (N. van Uden)
Metschnikowia (N. van Uden)

3. Publications.

Uden, N. van, "Factors of host-relationship". Proceedings VIII Int. Congr. Microbiol. Montreal 1962 (in press).

Uden, N. van, "On the nomenclature of the genus Metschnikowia Kamienski. Revista de Biologia 3: 95, 1962.

Buckley, H. and N. van Uden, "The identification of Candida albicans within two hours by the use of an egg-white slide preparation: Sabouraudia. (in press).

Abranches, P. and N. van Uden, "A method for the quantitative estimation of yeasts in sputum". Sabouraudia (accepted for publication).

Leiria, M. Vidal and N. van Uden, "Candida silvae nov. spec., a yeast isolated from humans and horses". Antonie van Leeuwenhoek (accepted for publication).

Uden, N. van and R. Castelo Branco, "Distribution and population densities of yeast species in Pacific water, air, animals and kelp off Southern California". Limnology and Oceanography (in press).

Uden, N. van and D. G. Ahearn, "Occurrence and population densities of yeast species in a fresh water lake". Limnology and Oceanography (accepted for publication).

Taysi, I. and N. van Uden, "Occurrence and population densities of yeast species in an estuarine-marine area". Limnology and Oceanography (accepted for publication).

4. FELLOWSHIP AVAILABLE:

During the period 1963-64 a Gulbenkian fellowship is available. Applicants should be willing to learn the methods of yeast identification and be interested to do research on marine yeasts, yeasts associated with animals or any other field of yeast ecology. Reasonable fluency in any one of the following languages is required: Portuguese, English, German, Dutch, Spanish or French.

IV. R. Inst. of Fermentation, Yamanashi University, Kitashin-machi, Kofu, Japan. Communicated by Dr. Shoji Goto.

The following two papers have been published in the Journal of the Institute of Fermentation of the University of Yamanashi. No. 9, pg. 88-99 and 99-106, Nov. 1962.

Abstracts

Studies on Japanese Wine Yeasts. Part 21. Taxonomic study on True Wine Yeasts which do not grow as a Film on the Surface of Wine or Culture Media containing Ethanol. By Isami Yokotsuka, Shoji Goto, and Shuki Morozumi.

76 typical strains of ascospore forming wine yeasts which, when seeded in grape juice of Koji extract, commenced growing as a deposit and brought about an alcoholic fermentation and then later did not grow as a film on the surface of wine or fermented media, were selected from 847 cultures of wine yeasts isolated from 470 wine samples collected at 66 wineries in grape and wine producing areas of Kofu valley, Japan.

They were classified in accordance with the procedures and systems of Lodder et Kreger-van Rij as follows:

27 strains of Saccharomyces cerevisiae Hansen; 9 of S. cerevisiae var. ellipsoideus (Hansen) Dekker; 11 of S. oviformis Osterwalder; 5 or S. bayanus Saccardo; 3 of S. heterogenicus Osterwalder; 5 of S. steineri Lodder et van Rij; 10 of S. rosei (Guill.) Lodder et van Rij; 4 of S. acidifaciens (Nickerson) Lodder et van Rij.

Taxonomic Studies on European Wine Yeasts. Isami Yokotsuka and Shoji Goto.

49 strains of European wine yeasts presented by three institutes in Germany, France and Portugal were taxonomically studied.

43 strains of them were identified with Saccharomyces consisting of 19 of S. cerevisiae, 8 of S. cerevisiae var. ellipsoideus, 3 of S. steineri, 5 of S. oviformis one of S. heterogenicus, one of S. chevalieri and 5 of S. italicus which have not been isolated in Japan.

The other six strains were identified with non-sporulating yeasts which consisted of four strains of Torulopsis colliculosa which were all flor yeasts, one of Candida tropicalis and one of Candida guilliermondii.

V. Southern Illinois University, Carbondale, Illinois. Communicated by Dr. C. C. Lindegren. (see also item VI).

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

1. Lindegren, C. C. The chromone theory of mitosis. Canadian Journal of Genetics and Cytology 4: 426-439 (1962).

2. Lindegren, C. C. Viruses, genes and cistrons. Nature 197: 566-568 (1963).

3. Pittman, D., Shult, E., Roshanmanesh, A. and Lindegren, C. C. The procurement of biochemical mutants of Saccharomyces by the synergistic effect of ultraviolet radiation and 2,6-diamino purine. Canadian Journal of Microbiology 9: 103-109 (1963).

4. Lindegren, C. C. Yeast Genetics 1962. The Brewers Digest. Vol. 38, No. 1, 43-53 (January 1963).

5. Lindegren, C. C. Directed mutations in yeasts and bacteria. Bulletin of the Research Council of Israel. Section A Chemistry, Volume 11A, Number 4, February 1963, pages 363-368.

VI. Southern Illinois University, Carbondale, Illinois. Communicated by Dr. Tadashi Hirano.

I am returning to Japan as Chief of Biology and Health Physics Division, Tokyo Metropolitan Isotope Center, Setagayaku, Tokyo, in July after spending four years in the United States where I worked as a Research Associate:Visiting Associate Professor in Professor Carl C. Lindegren's Laboratory (Biological Research Laboratory, Southern Illinois University, Carbondale, Illinois). Also, I had the opportunity to be in charge of the Electron Microscope Laboratory, Southern Illinois University, for a period of one year.

The cordiality and courtesy of the Southern Illinois University, particularly Prof. and Mrs. Carl C. Lindegren and Prof. H. M. Kaplan (Supervisor of Electron Microscope Laboratory) made my stay both enjoyable and scientifically rewarding.

The following papers have been published or submitted for publication:

1. Hirano, T., and C. C. Lindegren. Electron microscopy of the mitochondria in Saccharomyces. J. Ultrastruct. Res. 8, 322 (1963).

2. Hirano, T., and C. C. Lindegren. Electron microscopy of mitochondrial changes in Saccharomyces. J. Ultrastruct. Res. 8, 322 (1963).

3. Hirano, T., C. C. Lindegren, and Y. H. Bang. Electron microscopy of virus-infected yeast cells. J. Bacteriol. 83, 1363 (1962).

4. Hirano, T. The fine structure of the nuclear apparatus in Saccharomyces. J. Ultrastruct. Res. 7, 201 (1962) and in Electron Microscopy, Academic Press, N. Y. UU-4 (1962).

5. Hirano, T. Some intracellular relationships of the cytoplasmic membrane system of Saccharomyces. J. Ultrastruct. Res. (submitted).

6. Hirano, T., and D. Pittman. Electron microscopy of the protoplasts of Saccharomyces. J. Ultrastruct. Res. (submitted).

7. Lindegren, C. C., Y. H. Bang, and T. Hirano. Progress report on the zymophage. Transaction of New York Academy of Science 24, 540 (1962).

VII. Department of Radiation Biology, University of Rochester Medical School, Rochester 20, New York. Communicated by Dr. Fred Sherman.

The following articles have been published or will be published:

Sherman, F. and B. Ephrussi (1962) The relationship between respiratory deficiency and suppressiveness in yeast as determined with segregational mutants. Genetics 47: 695-700.

Sherman, F. and H. Roman (1963) Evidence for two types of allelic recombination in yeast. Genetics 48: 375-385.

Sherman, F. and P. P. Slonimski (in preparation) Respiration-deficient mutants of yeast. II. Biochemistry.

Slonimski, P. P. and F. Sherman (in preparation) A yeast mutant with an altered cytochrome c.

Sherman, F. (in preparation) Mutants of yeast deficient in cytochrome c.

VIII. Martin Luther University, Hautklinik, Halle/saale, West Germany. Communicated by Dr. Erika Friedrich.

The following monograph was recently published:

"Die Sprosspilze des Menschen, ihre Bestimmung mit Hilfe Morphologischer und biochemischer Methoden." (Published 1962 by Johann Ambrosius Barth-Verlag, Leipzig, 63 pages).

An abbreviated identification procedure is described for yeasts of medical importance. The methodology is based on the procedures given by Lodder and Kreger-van Rij in "The Yeasts". The simplification involves reliance on macroscopic morphology, as is done in bacteriology. The strains are separated into four different groups, based on colony morphology.

1st Group: colonies convex, cream-colored, smooth

2nd Group: Colonies smooth, glossy, low convex or flat, greyish-cream, slightly pigmented.

3rd Group: Colonies rough, truncate, low to high convex, greyish cream or whitish, folded or other surface structures.

4th Group: Colonies pigmented red.

Because of the small number of species, which according to general experience are found in clinical material, a more accurate diagnosis is already possible by doing only a few additional tests, after placing the organisms in the four groups listed above. For example, in a large number of such strains one can dispense with the trouble of looking for ascospores and also the assimilation tests are necessary for members of some groups only.

The methodology used, except for certain simplifications or expansions, is the same as that used in "The Yeasts".

Organisms which are further characterized are those which occur very frequently or moderately frequently in clinical material and those which can easily be confused with yeasts of medical importance because of morphologic and physiologic

similarities. These consist of 26 budding yeasts and Geotrichum candidum. The following reactions were tested:

Fermentation of sugars, assimilation of sugars and nitrate, macromorphology and micromorphology of strains grown on malt agar, ability to form ascospores and shape of the spores, if present. Black-and-white illustrations of colony-, cell- and ascospore shapes are given.

IX. Department of Enzymology, Instituto Municipal Centro de Investigaciones Biologicas, C.S.I.C., Madrid, Spain. Communicated by Dr. A. Sols.

After the successful resolution of the controversy on direct vs indirect fermentations of oligosaccharides into transport before (maltose, lactose) or after (sucrose, melibiose) splitting to monosaccharides (G. de la Fuente & A. Sols, Biochim. Biophys. Acta, 56: 49-62, 1962), work has been in progress in this laboratory on the specificity and kinetics of the constitutive hexose transport. Using a combination of approaches, including the photometric observation of the swelling of protoplasts, we have recently completed a detailed study of the substrate specificity of hexose transport in S. cerevisiae. More than a dozen sugars out of 40-odd examined have been found to be substrates with varying degrees of affinity for the transport. The rate of transport-mediated entrance of the best substrates (including the three fermentable hexoses glucose, fructose and mannose) at low concentrations, is a million times greater than that of simple diffusion across the membrane. Only trioses seem to enter readily by diffusion. Galactose is a very poor substrate of the constitutive hexose transport, but can enter readily through an inducible transport. L-Arabinose is a substrate for this inducible galactose transport but not for the constitutive one for hexoses.

A short communication on the "Role and formation of the acid phosphatase in yeast" has recently been published (C. F. Heredia, Fanny Yen & Alberto Sols, Biochem. Biophys. Res. Commun., 10: 14-18, 1963). In addition Dr. Heredia has found an inducible fructose diphosphatase in alcohol-grown yeast.

We are now carrying out a concentrated attack on the mechanisms of the Pasteur effect. Presently, it is recognized that none of the explanations so far advanced can fully account for the known facts. Preliminary evidence indicates that a series of two feed-back mechanisms link sugar entrance to overall utilization.

X. Seton Hall College of Medicine and Dentistry, Medical Center, Jersey City 4, New Jersey. Communicated by Dr. V. P. Cirillo.

The main interest in our laboratory continues to center on the mechanism of sugar transport. Our recent, unpublished work has been concerned with three main problems: (1) temperature effects on transport, (2) the Pasteur effect, and (3) the effect of polyene antibiotics.

Some experiments on the effect of temperature were reported at the A.S.M. meetings in Cleveland. Sugar transport was compared in baker's yeast and in an obligative, psychrophile, Candida #5, which grows well at 0°C and up to, but not higher than, 20°C. The main findings are (a) the rate of glucose metabolism, glucosamine accumulation (mostly as glucosamine-6-phosphate) and the transport of the non-metabolized, L-sorbose, for Candida #5 show a constant temperature coefficient (i.e. Q_{10}) of ca 2.0 over the whole range from 30° to 0°C. By contrast, these same processes in baker's yeast show a temperature coefficient of ca 3.0 from 30° to 10°, but more important, between 10° and 0° the Q_{10} is ca 20.0. Therefore, the psychrophile and baker's yeast differ mostly in the effect of temperature between 10° and 0°. Significantly, the activity of hexokinase in cell-free extracts of both yeasts is similarly affected by temperature showing a constant Q_{10} of ca 2.0 over the range

from 30° to 0°C. The relative insensitivity of the sugar transport system of the psychrophile in the range between 10° and 0°C is undoubtedly an important factor in its capacity to grow at 0°C. The corrallary of this observation is that the lower end of the temperature range of an organism may be set by the activity of its most temperature sensitive transport system.

During this work we found that 10^{-3}M uranyl nitrate at pH 4.0 is a useful agent in sugar transport experiments. The addition of uranyl nitrate to cell suspensions in sugar solutions immediately stops further transport. This has permitted closely timed samples to be taken in experiments where transport could not be stopped conveniently by immediate cold-washing. The intracellular sugars remain in uranyl blocked cells until washing can be carried out. If subsequent washing is done with 10^{-3}M uranyl nitrate solution less sugar loss occurs than washing with the usual ice cold, substrate-free medium. This is especially true in experiments with psychrophiles where sugar loss can be extensive without uranyl nitrate in the cold wash solution.

Our study of the Pasteur effect intends to determine whether this phenomenon in baker's yeast depends upon or results in a change in the rate of glucose transport in air versus nitrogen. This study is presently in the preliminary stage.

The results of our work with the polyenes, filipin and N-acetyl candidin, on sugar transport in baker's yeast have confirmed Lampen's conclusions on their mode of action at the cell membrane. Filipin, a representative of the small polyenes, causes complete loss of cell membrane integrity. This was determined by studying sorbose transport and competition with glucose. Since sorbose transport is a facilitated diffusion and, therefore, does not depend on energy metabolism, the complete loss of (1) sorbose retention during cold-water washing and (2) the complete loss of glucose competition for sorbose loss in exit experiments indicates that cell membrane integrity is destroyed by filipin.

N-acetyl candidin, a large polyene, again in confirmation of other work by Lampen, does only slight damage to cell membrane integrity using sorbose-glucose interactions as a criterion of membrane integrity.

Our work on the psychrophilic Candida has whetted our appetite to look at the sugar transport of other yeasts with unusual adaptations, i.e. thermophiles, halophiles, osmophiles and others. We welcome suggestions and cultures of such organisms which might provide useful information on the nature of the yeast cell membrane.

Recent Bibliography on Sugar Transport in Yeasts:

Cirillo, V. P. 1961 The mechanism of sugar transport into the yeast cell. Trans. N. Y. Acad. Sci., Sec. II, 23, 725-734.

Cirillo, V. P. 1961 Sugar transport in microorganisms. Ann. Rev. Microbiol., 15, 197-218.

Cirillo, V. P. 1961 Uranyl ion inhibition of sugar transport. Bacteriol. Proc., p. 92.

Cirillo, V. P. 1961 Transport of non-fermentable sugars across the yeast cell membrane. p. 343-351, in A. Kleinzeller and A. Kotyk (Ed.). Membrane Transport and Metabolism, Academic Press, Inc., New York.

Cirillo, V. P. 1962 Mechanism of glucose transport across the yeast cell membrane. J. Bacteriol., 84, 484-491.

Cirillo, V. P. 1962 Sugar transport by Saccharomyces cerevisiae protoplasts. J. Bacteriol., 84, 1251-1253.

KI. Department of Food Science and Technology, University of California, Davis. Communicated by Dr. J. F. T. Spencer and Dr. H. J. Phaff.

A summary of our ecological, taxonomic and physiological studies follows:

Of the yeasts isolated from flowers, more than half were strains of Pullularia. The remaining yeasts belonged to the genera Cryptococcus, Rhodotorula, Candida, Torulopsis, Sporobolomyces and Saccharomyces. Out of 80 isolates of Cryptococcus, only four were found to be Cr. laurentii, the remainder being Cr. albidus and Cr. diffluens. The remaining cultures were mostly Candida species (many of which are probably C. parapsilosis and C. krusei) and Torulopsis sp., six of the latter being producers of hydroxy fatty acid sophorosides. Some of the cultures of Candida and Torulopsis have presented some difficulties in identification and have not yet been classified. The Saccharomyces isolates include S. kluyveri, S. uvarum and S. rosei. Sporobolomyces roseus and Sp. pararoseus were found, and two cultures which resemble Ustilago zeae were found in Davis flowers.

Relatively more cultures of Cr. laurentii were found in the Cryptococcus isolates obtained from various water samples. Cr. albidus, Cr. diffluens, and Cr. neoformans var. uniguttulata were also isolated from water and from aquatic animals, but less often than from terrestrial sources. Rhodotorula macerans, R. glutinus, R. mucilaginosa, another Rhodotorula culture (which appears to be a new species), Candida catenulata, C. pulcherrima, Trichosporum cutaneum var. multisporum and a culture of Hansenula which has not yet been identified, were also found.

The Rhodotorula isolate, a rather light pigmented strain, was found to produce an unusually large proportion of γ-carotene, and traces of yellow carotenes. Production of torulin accompanies that of γ-carotene, but as the culture ages, the torularhodin concentration approaches that of torulin.

Some of the factors influencing the formation of a trisaccharide by Sporobolomyces singularis have been studied (cf. Phaff and do Carmo-Sousa, Antonie van Leeuwenhoek 28, 193, 1962). The yeast grows well in a medium containing lactose and yeast autolysate, but the trisaccharide is only produced in reasonable yields if the initial pH is adjusted to 3.5 - 4. At an initial pH of 4.0 small amounts of another oligosaccharide which may be a tetrasaccharide are produced. Increased aeration increased the yield of oligosaccharides. Several grams of the product have been recovered.

The carbon assimilation pattern of the Metschnikowia cultures isolated from brine shrimp of Manitou Lake, Sask., has been obtained. The yeast assimilates glucose, galactose, L-sorbose, maltose, sucrose, cellobiose, trehalose, D-mannitol, D-sorbitol, salicin, gluconolactone, and 2-keto-gluconate. Unlike M. zobellii, it does not assimilate melezitose, D-xylose, ethanol, adonitol, α-methyl glucoside or succinic acid. It differs from M. krissii in assimilating galactose, L-sorbose and sorbitol, and in not assimilating melezitose, ethanol, α-methyl glucoside and succinic acid. The new species ferments glucose only. A pseudomycelium is not formed on potato dextrose agar. The cells and asci are considerably larger than those of M. krissii and M. zobellii. Asci are usually 30-40μ in length. Each ascus contains a single needle-shaped spore. A similar organism was isolated from brine shrimp collected in the Black Sea in 1891 by Kamenskii, but cultures of it no longer exist.

Several new species will be described in detail in Antonie van Leeuwenhoek.

XII. Laboratory of Kodama Brewing Co. Ltd., Iizuka, Iidagawa, Showa-machi, Akita Prefecture, Japan. Communicated by Dr. K. Kodama.

The following is an abstract of a recently published paper:

Journal of Fermentation Technology Vol. 41, pg. 113-116, No. 3 (1963).
Studies on Wild Yeasts which Thrive in "Shubo" (I). Kenkichi Kodama and Tadashi Kyono.

It is well known that there are several kinds of microflora which appear to accompany "Sake" Yeast (Saccharomyces sake) during the preparation of "Kimotokei Shubo" (one of the early types of the starter which plays an important role in the cultivation of "Sake" yeast in the brewing of Japanese "Sake"). The flora change of wild yeasts seems to be an ecologically noteworthy phenomenon in connection with the purity of inoculated "Sake" yeast. This paper deals with 12 strains of wild yeast isolated from "Shubo" at the author's brewery. Our taxonomic studies revealed that all strains are quite similar to Candida guilliermondii (Cast) Langeron et Guerra in both fermentable sugars and assimilable carbon compounds, as well as in many of its morphological properties, except for a slight development of primitive pseudomycelium observed in some of the strains studied which is unlike the original description of C. guilliermondii. Further, in view of the fact that these yeast flora assimilate mannitol, sorbitol, glycerol and ethylamine in contrast to "Sake" yeast, these compounds should be considered as suitable carbon and nitrogen sources respectively in selective media employed for the determination of purity of "Sake" yeast in the final stage of "Shubo" preparation.

XIII. The Stroh Brewery Co., Detroit 25, U.S.A. Communicated by Dr. Philip E. Dakin.

The following is an abstract of a paper presented at the 29th Annual Meeting of the Am. Soc. of Brewing Chemists, Cleveland, Ohio, May 12-16, 1963. The full version of this paper will appear in the A.S.B.C. Proceedings, which are published in the Fall of 1963.

An Improved Yeast Counting Technique through the Use of the Microcentrifuge. F. Karadshi, J. Wilmot, P. Dakin.

The maintenance of the desired yeast levels throughout the brewing process is a key factor in the quality and character of the finished product, and the actual measurement of these levels becomes an important aspect of quality control in the brewery.

The estimation of total yeast cells in suspension using the haemocytometer has been considered the absolute standard for many years. However, as a control procedure, the haemocytometer method can be extremely time consuming.

The method which we will describe using the microcentrifuge has a precision comparable to the haemocytometer, and the additional advantage of providing reproducible results in a very few minutes. While the use of the microcentrifuge in the brewery may be novel, the instrument has found wide acceptance in the clinical field for some years for blood cell analysis.

The sample to be analyzed is well mixed to keep the yeast in even suspension. Replicate microcapillary tubes are filled by capillary action and plugged with clay. This is done simply by inserting the end of the capillary tube in the clay and rotating it slightly. The filled capillary tubes are then centrifuged for exactly 5 minutes at 15,000 RPM.

After centrifuging, the capillary tubes are placed in the Reader and the per cent yeast solids by volume are read directly from the scale. The scale on the reader is designed to compensate for variations in the total volume in the capillary tube and no initial measurements of volume or weight are required in the test.

Having obtained the haemocrit reading or per cent yeast solids from the Reader, the actual cell count in the original suspension can be read directly from a chart relating haemocrit reading to cell count.

To prepare the experimental curve, serial dilutions of several yeast suspensions were prepared and haemocytometer cell counts and corresponding haemocrit readings were made on each dilution.

We noted that the curve did not pass through the origin as one had expected. This deviation was probably due to suspended material other than yeast cells - most probably protein. To correct for this it would be necessary to treat the original suspension possibly with hydroxide to solubilize the protein material. Where the concentration of trub was high, it formed a distinct layer on top of the yeast and we were able to correct for this in the capillary tube Reader.

Where the cell concentration is low, possibly toward the end of the cellaring process, it may be necessary to pre-concentrate the yeast cells before running a haemocrit reading.

Although we have no experimental data to substantiate it, we feel for greatest accuracy, a separate curve should be prepared for each specific yeast strain.

In conclusion, the level of yeast cells in a suspension throughout the brewing process may be estimated rapidly and with precision using the microcentrifuge. The technique is simple and as it requires a minimum of skill, is well suited for use in the plant. The microcentrifuge may prove to be a useful tool in yeast analysis not only in the laboratory but in the plant as well.

XIV. Brief News Items.

1. My colleague, Rosalinde Hurley and I have almost completed our Monograph on Candida albicans. We are continuing our studies on the pathogenicity of various members of the Candida group. We are also studying the ultrastructure of Candida albicans as revealed by the Electron Microscope. Papers on the pathogenicity of C. tropicalis and on renal moniliasis have recently been presented to the Pathological Society of Great Britain and Ireland. A paper on Candidosis of the lung was presented at the 8th Congress of Microbiology at Montreal last year.

I shall be very happy to see any Mycologists who might be visiting London if they would care to come to the Department of Bacteriology, Charing Cross Hospital Medical School, W.C.2. (Telephone TEMple Bar 7788).

H. I. Winner

2. On December 1, 1962, Miss Charlotte C. Campbell was appointed Associate Professor of Medical Mycology, Harvard University School of Public Health, Boston, Mass., after twenty-one years at the Walter Reed Army Institute of Research, Washington, D. C. For the latter fifteen of these she was Chief of the Medical Mycology, Department of Bacteriology.

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One Shattuck Street
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3. Mr. James Middlekauff has recently begun Ph.D. research in my laboratory, and hopes to work on the genetics of mating type in Hansenula wingei.

T. D. Brock has given a paper on the molecular basis of mating in yeast at a symposium of the New Jersey branch of the A.S.M. on the biology of yeasts. He will give a lecture in the summer course on experimental embryology at Woods Hole on the surface properties of microorganisms associated with cell-to-cell contact and interaction.

Thomas L. Brock
Department of Bacteriology
Indiana University
Bloomington, Ind., U.S.A.

4. We are currently investigating microorganisms, including yeasts, associated with the sugar beet before and after harvesting and under various climatic conditions, such as freezing.

We should be pleased to correspond with any reader of the "News Letter" who may be engaged on work covering the presence of yeasts in undamaged fruits or plants, particularly root crops.

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Peterborough, Northants, England

5. The following article will soon appear "Les levures à spores verruqueuses" by F. Abadie, M. C. Pignal and J. L. Jacob.

We shall be glad to exchange reprints with interested colleagues. We are preparing an article on the yeasts from the Cameroun: "Several yeasts from the Cameroun". Few sporogenous yeasts,(Pichia silvestris, Guilliermondella lodderi, Sacch. steineri and S. cerevisiae) and numerous Candida species among which two new species.

M. C. Pignal
Laboratoire de Microbiologie et Mycologie
Faculté des Sciences de Lyon
16, Quai Claude-Bernard
Lyon 7e - France

6. The following two papers may be of interest to readers of the Yeast News Letter:

(In Preparation)
"List of Cultures" of yeasts and molds maintained in our laboratory: The list will be distributed to laboratories dealing with the maintenance of microorganisms and anyone interested in receiving it.

(In Press)
"Preservation of microorganisms with a simplified lyophil process". N. Kawakami, O. Niimi, H. Maeda, A. Gotanda, M. Oda and T. Nehira (1963): Bulletin of the Faculty of Engineering, Hiroshima University.

Noboru Kawakami, Dr. Engineering
Department of Fermentation Technology
Faculty of Engineering
Hiroshima University
Hiroshima, Japan

7. The following paper has been published in the Journal of Applied Bacteriology 25, 202-212 (1962).

The Mineral Nutrition of Hansenula spp. of Yeast in Relation to Fat Production. By T. K. Fahmy, J. W. Hopton and M. Woodbine, Microbiological Unit, Department of Agricultural Sciences, University of Nottingham, England.

SUMMARY: The development of cultures of 'high fat' and 'low fat' of Hansenula under different nutritional regimes in shaken culture has been examined. The cultures were sampled at frequent intervals throughout growth, and determinations made of cell number and cell mass, uptake of nitrogen and glucose and synthesis of fat and polysaccharide.

In all media there was an initial phase of rapid multiplication associated with nitrogen uptake, but appreciable increase in yeast mass occurred after the glucose had been exhausted and most of the accretion of fat and polysaccharide occurred during this phase of growth. In a nitrogen deficient medium the production of fat and polysaccharide was accentuated and, whereas the cell count of 'low fat' species increased during the second assimilatory phase, that of 'high fat' species did not. The behaviour of yeasts in a phosphorus deficient medium simulated that in a nitrogen deficient medium, but behaviour in a medium containing a low concentration of sulphur differed little from that in a complete medium.

Dr. M. Woodbine

8. The following booklet has been published:

"Studies on the lipid-forming yeast Cryptococcus terricolus nov. sp. by T. A. Pedersen, Dept. of Microbiology, Agric. College of Norway, Vollebeck, Norway. 1963, 176 pages.

Individual chapters have been published in the following journals:

1. Cryptococcus terricolus nov. spec., a new yeast isolated from Norwegian soils. C. R. Lab., Carlsberg 31, 93-103, 1958.

2. Studies on the physiology of the soil yeast Cryptococcus terricolus. Physiol. Planterum 13, 64-75, 1960.

3. Lipid formation in Cryptococcus terricolus. I. Nitrogen Nutrition and Lipid Formation. T. A. Pedersen, Royal Institute of Technology, Div. of Food Chemistry, Stockholm 70, Sweden. Acta Chemica Scandinavica 15 (1961) 651-662.

4. Lipid Formation in Cryptococcus terricolus. II. The Effect of Ion Variation on Growth and Lipid Formation. T. A. Pedersen, Royal Institute of Technology, Div. of Food Chemistry, Stockholm 70, Sweden. Acta Chemica Scandinavica 16 (1962) 359-373.

5. Lipid Formation in Cryptococcus terricolus. III. Extraction and Purification of Lipids. T. A. Pedersen, Royal Institute of Technology, Division of Food Chemistry, Stockholm 70, Sweden. Acta Chemica Scandinavica 16 (1962) 374-382.

6. Lipid Formation in Cryptococcus terricolus. IV. Separation of the Lipid Extract by Silicic Acid Column Chromatography. T. A. Pedersen, Royal Institute of Technology, Div. of Food Chemistry, Stockholm 70, Sweden. Acta Chemica Scandinavica 16 (1962) 1015-1026.

7. Lipid Formation in Cryptococcus terricolus. V. Oxidation of Fatty Acids by Cell Suspensions. T. A. Pedersen, Dept. of Microbiology, Agricultural College of Norway, Vollebekk. Physiologia Plantarum, Vol. 16, 1963.

8. Lipid Formation in Cryptococcus terricolus. VI. Effect of Malonate on Respiration. T. A. Pedersen, Dept. of Microbiology, Agric. College of Norway, Vollebekk. Physiologia Plantarum, Vol. 16, 1963.

9. Universal Foods Corp. (Red Star Yeast Co., Milwaukee, Wisc., U.S.A.) reports:

New Yeast Developed

United States winemakers will get a helping hand from the Laboratory of Microbial Chemistry, a part of the Corporation's research laboratories. A new compressed "wine" yeast has been developed and is now being used by some West Coast and Chicago winemakers. Red Star is the first company to develop and market this new product which is used to ferment grape sugars to alcohol for wine.

Before the introduction of this yeast, winemakers had to grow their own yeast. Now there is a better yield of wine with the new yeast, because some grapes no longer have to be used for this purpose. The "wine" yeast also gives more efficient fermentation.

The more efficient fermentation and the higher yield could tend to lower the production costs for winemakers who use Red Star's new compressed yeast for wine.

According to Gordon R. Christensen, director of Red Star Yeast Industrial Sales Division, Red Star, in cooperation with a West Coast winemaker, worked over a year and a half developing this special strain of yeast. This yeast is available to all the wine industry.

10. In November of 1961 I have donated to the Faculty of Chemistry, Montevideo, Uruguay, my private collection of microorganisms. This collection consists of several hundred strains of yeasts and molds. The collection new remains in care of the laboratory under the direction of Professor C. Cano Marotto, to whom inquiries should be directed. I wish to thank at this time those scientists who have contributed cultures to my collection.

R. C. Artagaveytia-Allende
Fac. de Chimica
Av. General Flores 2124
Montevideo, Uruguay

XV. Letters to the Editor:

Dear Sir,

The two principal breeding stocks of yeast are those from Winge's and from our laboratory. Winge's gene-markers involve almost exclusively carbohydrate fermentation; nearly everyone using nutrilite deficiencies got their original stocks from us or from workers who obtained cultures from us and then distributed them to others. Often the markers have been renamed, as might be expected in the case of anthranilic- and tryptophane-deficient cultures. Many users of these stocks are unaware of their origin and have spoken of the cultures as Saccharomyces cerevisiae. (F. Rudert and H. O. Halvorson. The effect of gene dosage on the level of alpha glucosidase in yeast. Bulletin of the Research Council of Israel, Vol. 11A, No. 4, pages 337-344, February 1963. D. C. Hawthorne and R. K. Mortimer. Super-suppressors in yeast. Genetics, Vol. 48, No. 4, pages 617-620, April 1963). I dwelt for some time in my book on the fact that this designation cannot be used because many species other than cerevisiae were incorporated into the stock. It is wrong to call these cultures Saccharomyces cerevisiae when they should be spoken of simply as the

Lindegren Saccharomyces Breeding Stock.

Carl C. Lindegren, Director
Biological Research Laboratory
Carbondale, Ill., U.S.A.

Dear Sir,

At the Robert A. Taft Sanitary Engineering Center Fungus Studies Laboratory, we are trying to determine what yeasts occur in the environment of polluted water. A recent project, summarized in "A study of yeast populations in a waste stabilization pond system" published in the Hofler Festschrift volume of Protoplasma in May, was based on the fungi, including yeasts, present in that type of sewage treatment system. We are trying to develop a method of enumerating the yeast populations of a polluted or an unpolluted habitat. In no one set of isolation techniques can we get all the species of yeasts present in a population or a sample, but for that segment isolated by the technique being used we can arrive at a fair notion of the abundance of or lack of yeasts. It is beginning to look as if in a habitat in which there is organic enrichment of one sort or another yeasts are more abundant than in habitats without such enrichment. Because the presence of smut-like yeast-like isolates in a habitat may be of interest in studies which may be developed in the future, it is requested that such cultures not be discarded. If such cultures are available I should like to know about them and I should like to have some representative isolates.

Wm. Bridge Cooke
Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio, U.S.A.

Dear Sir,

JFCC, or the Japanese Federation of Culture Collections of Microorganisms, organized in 1951 by the recommendation and under the joint sponsorship of the Ministry of Education and the Science Council of Japan is a federation of type culture collections in Japan which is actively engaged in collection, preservation, distribution and exchange of type cultures of microorganisms.

The chief object of this federation is to encourage on a global basis type culture collection and distribution through close cooperation of various interests involved in type culture collections, and, by so doing, to contribute to the studies of microorganisms and their application.

The first president was Dr. Yasuhiko Asahina, followed by Dr. Takeo Tamiya, Dr. Kiyoshi Kominami, Dr. Kin-ichiro Sakaguchi and Dr. Toshinobu Asai. Dr. Masashiro Kudo is the president of JFCC at present.

The present organization is as follows:

Name: Japanese Federation of Culture Collections of Microorganisms

Office: c/o Institute for Infectious Diseases, University of Tokyo, Shiba-shiroganedai-machi, Minato-ku, Tokyo

President: Dr. Masashiro Kudo (Inst. Infectious Diseases, Tokyo Univ.)

Vice President: Dr. Hiroshi Iizuka (Inst. Applied Microbiology, Tokyo Univ.)

Secretary: Dr. Shizuo Momose (Higher Education and Science Bureau, Ministry of Education)
Dr. Takezi Hasegawa (Inst. for Fermentation, Osaka)
Dr. Kazuo Sato (Inst. Infectious Diseases, Tokyo Univ.)
Mr. Hiroshi Akamatsu (Inst. Applied Microbiology, Tokyo Univ.)

Advisor: Dr. Yasuhiko Asahina
Dr. Ryoji Nakazawa
Dr. Takeo Tamiya
Dr. Koji Ando
Dr. Kiyoshi Kominami
Dr. Kin-ichiro Sakaguchi
Dr. Hirosuke Naganishi
Dr. Kikichi Sato
Dr. Toshinobu Asai

JFCC: Catalogue of Cultures. 1962

In 1951 the Higher Education and Science Bureau of the Ministry of Education collected catalogue of stock cultures of microorganisms which were preserved in various research institutions throughout Japan. Based upon this material, Dr. Kiyoshi Kominami compiled "A General Catalogue of the Cultures of Microorganisms maintained in the Japanese Collections", which was published in 1953. In the catalogue about 22,300 strains out of 144 institutions were listed, covering a majority of strains isolated and studied in the past 60 years by a host of investigators of microorganisms in Japan, in addition to those sent from abroad.

However, some of them were either identified a long time ago or transplanted over a period of time, so that a need has been felt to re-identify those strains listed in the catalogue under the more modern taxonomical criteria. Thus, with the cooperation of about 40 specialists of each group of microorganisms, "The cooperative research in re-identification and classification of microorganisms preserved in various institutions of Japan" was organized, headed by Dr. K. Sakaguchi. As a result, a majority of the strains preserved in various type culture collections connected with JFCC was confirmed their identity or re-identified to proper species. And these strains occupy the important part of this new catalogue.

"JFCC: Cagalogue of Cultures. 1962" (p. 282) is available at $4.00 (U.S.A. dollar, postage included), sent on receipt of payment.

Mail Transfer preferred through THE DAI-ICHI BANK, LTD., Hongo Branch, Tokyo. Account No. 60,094. Mr. Hiroshi Akamatsu. Pay on Tokyo.

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