Y E A S T
A News Letter for Persons Interested in Yeast

November 1960 Volume IX, Number 2

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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 May 1961. A contribution of $0.50 from those who have not contributed for some time would be appreciated to finance the 1961 editions of the News Letter. Many thanks to those who have contributed recently.

The Editors

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

The following new species (for which a description has been ~~published~~) have been received by the C. B. S. since publication of the last issue of the Yeast News Letter.

Candida fimetaria Soneda var. diversa Ohara, Nonomura et Yunome (Y. Ohara, H. Nonomura and H. Yunome, J. Agr. Chem. Soc. Japan, 34 : 709, 1960).
Candida vinaria Ohara, Nonomura et Yunome (Y. Ohara, H. Nonomura and H. Yunome, J. Agr. Chem. Soc. Japan, 34 : 709, 1960).
Cryptococcus ater Castellani (A. Castellani, J. Trop. Med. Hyg., p. 27, 1960).
Hansenula polymorpha Morais et Maia (J. O. Falcão de Morais e M. H. Dalia Maia, An. Esc. Sup. Quim. Univ. Recife, 1, 15, 1959).
Saccharomyces carlsbergensis Hansen var. alcoholophila Shehata (A. M. El-Tabey Shehata, Appl. Microbiol., 8, 73, 1960).
Schwanniomyces alluvius Phaff, Miller et Cooke (H. J. Phaff, M. W. Miller and W. Bridge Cooke, Antonie van Leeuwenhoek, 26, 182, 1960).
Sporobolomyces marcillae Santa Maria (J. Santa Maria, Arch. Mikrobiol., 32, 29, 1958).
Torulopsis bacillaris (Kroemer et Krumbholz) Lodder var. obesa Ohara, Nonomura et Yunome (Y. Ohara, H. Nonomura and H. Yunome, J. Agr. Chem Soc., Japan, 34 : 709, 1960).
Torulopsis domercqii v.d. Walt et v. Kerken (J. P. van der Walt and A. E. van Kerken, Antonie van Leeuwenhoek, 26, 314, 1960).
Torulopsis saccharum Shehata (A. M. El-Tabey Shehata, Appl. Microbiol., 8, 73, 1960).

Publications:

1. "Zur Systematik einiger Cerambycidensymbionten", G. Jurzitza, H. Kühlwein and N. J. W. Kreger-van Rij, Arch. Mikrobiol., 36, 299, 1960.
2. "Cryptococcus neoformans and its variety uniguttulatus", by N. J. W. Kreger-van Rij, Antonie van Leeuwenhoek (in press).

II. Noda Institute for Scientific Research, Noda 399, Noda City, Chiba-ken, Japan. Communicated by Dr. Hiroshi Onishi.

I have isolated an obligate halophilic yeast from old soy sauce mash and named it Torulopsis halonitratophila nov. sp.

As far as bacteria are concerned, a number of microorganisms which are obligately halophilic have been recognized. However, no obligate halophilism has yet been found in yeast, only salt-tolerant types being so far described.

It is of particular interest that though Torulopsis halonitratophila shows obligate halophilic property at 30°C it exhibits a facultative halophilism when tested at a lower temperature such as 20°. As a conclusion of this observation, it should be emphasized that cultural

temperature is the most important factor for halophilism.

Reference : H. Onishi, Bull. Agr. Chem. Soc. of Japan, Vol. 24, No. 3, 226 (1960).

III. Department of Microbiology, Queen's University of Belfast, Northern Ireland. Communicated by Dr. D. W. R. Mackenzie.

Factors affecting the early morphogenesis of pathogenic yeasts are being studied, using air bubble and tissue culture techniques, and animal experiments. In Candida albicans a "germ tube" is very rapidly formed and within the first 4 hours in vivo growth is exclusively concerned with cell elongation. C. tropicalis was also found to produce marked pseudomycelial formation in the first 4 hours within subcutaneous air bubbles in mice. In vitro, cells of C. albicans form "germ tubes" indistinguishable from those formed in vivo, in the presence of 2% ox serum. Studies are in progress concerning the role host immunology plays in determining the morphology of pathogenic fungi.

A new orange-coloured, capsulated yeast isolated from the atmosphere is being studied and will be reported as a new species.

"Yeasts isolated from Man" - the result of a survey of human sources is being published in "Sabouraudia". Of interest was the high incidence of Torulopsis glabrata which was second only to C. albicans in the survey, and the most common species isolated from urine.

IV. Istituto di Microbiologia Agraria e Tecnica della Universita di Pisa (Italy). Communicated by Professor O. Verona.

1. O. Verona and G. Picci are going forward in their researches on soil yeasts. These researches have an ecological aim, with particular reference to the finding of species of Lipomyces. Such species preferably occur in slightly acid and relatively ferrous soils. The presence of Lipomyces is of a certain ecological interest as (1) species of this Genus are N-fixing and (2) being mucogenous, contribute to soil structure formation. Hansenula saturnus and Candida guilliermondii were very frequently isolated from soils from different parts of Europe. Moreover, several strains of Schwanniomyces, referable to S. occidentalis and S. castellii, were isolated. One strain of Schwanniomyces (kindly controlled by Mrs. Kreger-v. Rij) was referred to S. alluvius, so this species is also present in European soils. About S. occidentalis, S. castellii and S. alluvius, being all species occurring in soil and having some similar characters, Verona and Picci studied modifications eventually arising on these species after remaining in soil for a long time. Important is to establish their limits of variability and consequently the validity of such species, as they are similar in many features.

2. Verona emphasizes that species containing large oildrops are assimilating atmosphaeric nitrogen. Such was found in Candida pulcherrima.

3. Cultivating yeasts on sucrose bean agar, with L-tryptophane present, Verona and Picci noticed that a deeply brown pigment is formed, which diffused in the medium. This fact was observed

for Aerobacterium liquefaciens and, even before, for some strains of Pseudomonadaceae. Among yeasts, it especially occurs in species of Saccharomyces, Pichia, and for Trigonopsis variabilis, Sporobolomyces salmonicolor and Rhodotorula mucilaginosa, at least for the strains tested. For Candida the pigment is generally absent or weakly present; uncertain for Debaryomyces. The study of the correlations existing between this activity and the others which characterize Genera and species, and above all, the nature of the pigment, are viewed as very interesting arguments.

4. Dr. Rambelli is studying yeasts occurring in flowers of Eucalyptus. He already isolated many species, one of which occurred very frequently, but not yet determined.

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

Visits and Changes

Dr. N. van Uden returned last September from a nine months' trip to the U.S.A., which he describes in high tones. He spent most of his time fishing yeasts from the Pacific and the Atlantic. The well remembered bases of operation were Dr. Claude E. ZoBell's laboratory (Scripps Institution of Oceanography, La Jolla, Calif.) and Dr. Samuel P. Meyer's laboratory (Marine Laboratory, Miami, Florida). Other highlights were the Second Conference on Medical Mycology, where he presented a yeast paper (The occurrence of Candida and other yeasts in the intestinal tract of animals. Annals of the New York Academy of Sciences. 1960. 89 : 59-68) and short visits to the laboratories of Dr. Emmons (Bethesda, Md.), Dr. Wickerham (Peoria, Ill.), Dr. Phaff (Davis, Calif.), Dr. Oppenheimer (Port Aransas, Texas), Dr. Roth (Miami, Florida), Dr. Ajello (Atlanta, Ga.).

Miss Lidia do Carmo Sousa, D.V.M., who worked during five years in this laboratory is now spending a year in Dr. Phaff's laboratory; everybody here is praying that she will not be lost to us forever.

Mr. R. Castelo Branco D.V.M., who had already been with this group for some months on an earlier occasion, will now work here full time for a yet indefinite period. He is studying fungi of interest in veterinary pathology and is collaborating with Dr. van Uden in the identification of yeasts from the Pacific Ocean.

Mr. A. Correia, a student of biology, also joined our group. He is now looking for Cryptococcus neoformans and other yeasts in pegeon manure from the many public places of Lisbon with large pigeon populations.

Fellowships

During 1961-62 we probably will have fellowships available for Portuguese and foreign students, interested to:

1. Practice yeast identification.
2. Do research work on yeasts associated with warmblooded animals or marine yeasts or any other field of yeast ecology.

Inquiries from qualified persons are invited. Reasonable fluency in any one of the following languages would be helpful: Portuguese, Spanish, French, English, German or Dutch.

Request.

We should like to obtain the addresses of the following yeast authors. Readers who find their or their friends' names in the following list are hopefully requested to drop us a line: R. Bahadur; Clark (Candida malicola); Florenzano; M. Giovanozzi; K. E. Graebner; Kobayashi; A. Kockova-Kratochvilova; P. Lavie; Y. S. Lewis; P. J. Luteraan; Malan; M. Mogi; Nakazawa; Y. Ohara; Y. Otani; P. Sonne Frederiksen; Suminoe; Tatsumi; Tubaki; Yamaguchi; Yamazaki; Yokotsuga; L. S. Walters; Zenitani.

VI. Muséum National D'Histoire Naturelle, Parc Zoologique, Paris-XII. Communicated by Dr. Henri Saëz.

My work is concerned with researches on the saprophytic and parasitic fungi occurring on the animals living in the Zoological Garden of Paris.

Publications:

1. Henri Saëz, Champignons isolés chez des flamants et des pelicans morts en captivité. Les Cahiers de Médecine Vétérinaire. Septembre-Octobre 1960, Volume XXIX, no. 5.
2. Henri Saëz, Le Geotrichum candidum Link, hote fréquent du tube digestif de quelques animaux sauvages en captivité. Bulletin de la Société Mycologique de France, Tome LXXV, Fascicule 2 de 1959. 170-176.
3. Henri Saëz, Contribution a l'étude de la mycoflore intestinale des animaux sauvages en captivité. Revue de Mycologie, Tome XXIV, Fasc. 5, 426-433, 1959.
4. Henri Saëz, Étude de l'utilisation de composés carbones par quatre especes de Geotrichum: G. candidum, G. pulmoneum, G. redaellii et G. asteroides. Extrait du Bulletin Mensuel de la Société Linnéenne de Lyon 29, 96-98, 1960.
5. Henri Saëz, Flore fongique intestinale du manchot royal, Rec. Méd. Vét. Tome CXXXVI (Juillet 1960), 567-573.

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

1. Dr. Yoneyama will return to Hiroshima University on January 2, 1961 after a stay of 15 months at Davis. A paper describing a new species of yeast, Endomycopsis scolyti, will be published in the journal Antonie van Leeuwenhoek. The natural habitat of the yeast is in frass or larvae of Scolytus ventralis and Scolytus unispinosus. Both are bark beetles which attack Abies and Pseudotsuga. Occasionally it is found associated with other bark beetles (Dendroctonus or Ips). The yeast has a wide geographic distribution. A study is underway of the yeast population in an active slime flux of Ulmus caprinifolia growing in Davis. The flux is sampled every two weeks throughout the year and the yeast population estimated quantitatively. The principal yeasts present are Geotrichum, Pichia pastori and one or more species of Prototheca. Seasonal variations in total and

relative numbers have been observed.

2. A project dealing with bacterial enzymes able to digest cell walls of yeast (with Mr. H. Tanaka) is being continued. We have prepared a partially purified enzyme from a species of Bacillus which is able to break down the glucan component of walls of various species of yeast. Living cells of yeast are not attached by the enzyme, but ascus walls of many species are readily digested. In this connection it is a useful aid in the dissection of asci. It has certain advantages over the digestive juice of snail, since the bacterial enzyme lacks mannanase and the resulting spores are held together in groups of four by the residual mannan. Only in the case of the yeast-like fungus Ashbya have we been able to produce protoplasts from living young hyphae (with the participation of Mrs. Lynn W. Higgins). The protoplasts are usually produced from the tips of young hyphae. Certain phases of this work will be reported at the next annual meeting of the Society of American Bacteriologists in Chicago (April 1961).

3. Miss L. do Carmo-Sousa D.V.M. from Lisbon, Portugal arrived in Davis October 1 to spend a year in our laboratory. One of the projects to be studied deals with the yeast flora found in a number of species of Drosophila, which occur in the coastal mountains of California.

4. Publications:

M. W. Miller, H. J. Phaff and H. E. Snyder. "On the occurrence of various species of yeast in nature". Mycopathologia et Mycologia applicata (accepted for publication).
R. L. Kitchel and M. W. Miller "The viability of a yeast in high density orange concentrates". Food Technology 14, 547, 1960.

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

We have succeeded to check polyploid yeasts for their ploidy by our mass isolation technique. The observed segregation ratios agree well with the theoretical numbers. It could be proved that one yeast was triploid $a^{\alpha}a^{\alpha}a^{\alpha}$ and the other tetraploid $a^{a}a^{a}a^{\alpha}a^{\alpha}$. (C. C. Emeis and S. Windisch: Prüfung der Kernwertigkeit polyploider Hefen mit der Massenisolationstechnik (short communication); Naturwissenschaften 47, 210/11, 1960.)

The ecological investigations on the yeast flora of beers have been continued. The figures at our disposal (received from qualitative and quantitative biological analyses of 206 Pilsen beers, 42 light beers and 75 light export beers) made it possible to arrange the yeasts and molds we found according to frequency and quantity. Examples are given for the assessment of ecological analyses for the control of brewing operation. The yeasts show an evident gradation of their frequency. 35 species were found. (S. Windisch: Über die Hefeflora unserer Biere; Monatsschrift für Brauerei 13, 53-59, 1960.)

The sugar Palatinose (α-Glucosido-6-fructofuranose), which has been discovered by Weidenhagen and Lorenz, can be fermented by some yeasts. The Palatinose fermentation has been checked by genetically well defined strains of Saccharomyces carlsbergensis. The Palatinose fermentation which is governed by only one gene is independent of Maltose and Saccharose fermentation. (C. C. Emeis and S. Windisch: Palatinose-vergärung durch Hefen; Zeitschrift für die Zuckerindustrie 10, 248/49, 1960.)

The handbook "Die Hefen", issued by Reiff, Kautzmann, Lüers and Lindemann, of which the 1st volume came out in August, contains chapters by S. Windisch and W. Laskowski. Those have come out as a reprint "Die hefeartigen Pilze", publisher: Hans Carl, Nürnberg 1960. The reprint includes the pages 23 - 208 of the 1st volume of the handbook. Publisher's price of this reprint is 24 German Marks.

I would like to ask the readers of the Yeast News Letter to send me strains of Saccharomyces pastorianus which will surely ferment 2/3 of Raffinose.

IX. Department of Fermentation Technology, Faculty of Engineering Osaka University, Osaka, Japan. Communicated by Dr. Y. Oshima.

Dr. Okada and I are working on the biochemical genetics of alpha-glucosidase formation in Saccharomyces under Professor G. Terui. Recently, I published an article concerning the genetic analysis of maltose fermentability of our test strains.

"Genetic studies on the fermentability of saccharomyces yeasts; on the fermentability of maltose" by Yasuji Oshima, Jour. Ferm. Technol., 38, 521-531 (1960).

Abstract. Genetic analysis of polymeric genes for maltose fermentability was undertaken employing a diploid hybrid D-346 (cf. Terui, Okada and Oshima: Technol. Rept. Osaka Univ., Vol. 9, 237, 1959).

The maltose genes of this strain could be analyzed into three genetic factors, in which MAα and MAγ were proved to be individual genes. But with another genetic factor, MAβ, the feature of segregation indicated that it would be composed of complementary genes. After a number of crosses between MAβ haploids having no other maltose genes and maltose-negative haploids, intensive tetrad analysis was performed. The results led to the indication that not less than three complementary genes are involved in the genetic factor MAβ.

On the physiological studies of our test strains, Dr. Okada has examined in detail the kinetics of 3 different maltases which are controlled in cellular production by different genetic systems, namely MAα, MAβ and MAγ, and he also examined 2 isomaltases which are controlled by different alpha-methyl-glucoside genes Y_1 and Y_2.

We observed apparent differences among those 5 alpha-glucosidases which are controlled by different genes, especially on the ratios of maximum velocity in hydrolyzing maltose, alpha-methyl-glucoside and alpha-phenyl-glucoside as substrates of those alpha-glucosidases.

X. Institute of Animal Genetics, West Mains Road, Edinburgh 9, Scotland. Communicated by Dr. C. H. Clarke.

As part of Dr. Charlotte Auerbach's project on chemical mutagenesis, I am studying auxotrophs of Schizosaccharomyces pombe (obtained from Dr. Urs Leupold and Mr. R. B. Drysdale). In the adenine and histidine-requiring double mutant ad. 1-3, his. 2-73, h^+, the adenine site responds to U.V., di-epoxybutane, ethylene imine, β-propriolactone, H_2O_2 + HCHO, and HNO_2. The histidine site, however, appears to be mutagen stable, though there is a slight response to HNO_2. It is planned to test further mutagens, in an effort to find conditions under which this histidine site will revert to histidine-independence.

Further tests on mutagen specificity are being carried out on double mutants in which ad. 1 sites are combined with a mutable lysine, methionine, or leucine marker.

XI. Southern Illinois University, Carbondale, Illinois. Communicated by Dr. C. C. Lindegren.

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

1. Lindegren, Carl C. An alternative theory of DNA action. J. Theoretical Biology. Accepted for publication.
2. Desborough, Sharon, Shult, E. E., Yoshida, T. and Lindegren, C. C. Interference patterns in Family Y-1 of Saccharomyces. Genetics. Accepted for publication.
3. Lindegren, Carl C. Gene controlled resistance to poisons in Saccharomyces. Proc. Society of Industrial Microbiology "Conference on Anti-microbial Agents," Washington, D. C. (Oct. 26-28, 1960).
4. Lindegren, Carl C. and Bang, Y. N. The zymophage. I. The demonstration of infectivity. Antonie van Leeuwenhoek. Accepted for publication.

Dr. Shizu Washio, Professor at the Atomi Junior College in Japan, is spending a year in the Biological Research Laboratory.

Dr. Lindegren received a Graduate Training Grant from the National Institutes of Health, Public Health Service for a five-year period. We are prepared to offer fellowships for Ph.D. candidates in yeast genetics. We have more than 50,000 genetical cultures identified with more than 30 carbohydrate, purine, pyrimidine, vitamin, amino acid, poison and antibiotic markers. Twenty to 30 markers can be incorporated into a single hybrid. About 9 to 10 chromosomes have been identified. We are equipped with electron and ultraviolet microscopes, micromanipulators, and other accessory equipment. Yeast is an ideal organism for the study of gene mutation, gene conversion, linkage and chromosome mapping, crossing over and interference, biochemical pathways and biochemical interference.

XII. Microbiology Laboratory, Heriot-Watt College, Edinburgh, Scotland. Communicated by Dr. A. H. Rose.

Mr. Fazal Ahmad is continuing his studies into the metabolic function of biotin in Saccharomyces cerevisiae. He is currently examining the

biotin-sparing effect of various purines, pyrimidines, nucleosides, nucleotides, and amino acids, and the effect that these substances have on the DNA, RNA, protein and ATP contents and intracellular amino acid pool in yeast. Mr. Ahmad should complete his studies in October of this year. Several other facets of this problem are also being studied in this laboratory. Mr. John Dunwell is interested in the polysaccharide composition of biotin-optimal and tiotin-deficient S. cerevisiae; he is particularly interested in the composition of biotin-deficient yeast grown in media containing L-aspartate for, under these conditions, the yeastcells remain clumped and fail to separate. The effect of biotin deficiency on the activity of various enzymes in S. cerevisiae is occupying the attention of Mr. Derek Mitchell.

Another group in this laboratory is working on the biochemistry of psychrophilic yeasts. Mr. Per-Otto Hagen has been working with a psychrophilic species of Cryptococcus (apparently a previously undescribed species) isolated by Dr. W. J. Nickerson in Labrador, and Mr. K. McRae is looking into methods of obtaining cell-free extracts of this yeast. Mr. H. McGill is studying the composition and structure of a polysaccharide produced at around 0°C (but not at higher temperatures) by another psychrophilic yeast kindly made available to us by Dr. R. P. Straka. If any readers of the Yeast News Letter should have cultures of psychrophilic yeasts, we, in this laboratory, would very much appreciate receiving subcultures.

The following publications have appeared from this laboratory during the past year:

A. H. Rose, Yeasts. Scientific American (1960) 202, 136.

A. H. Rose, Microbes in Industry. Science News (1960) No. 54, 67.

A. H. Rose, Excretion of nicotinic acid and nicotinic acid adenine dinucleotide by biotin-deficient yeast. Nature (London) (1960) 186, 139.

A. H. Rose, Excretion of nicotinic acid by biotin-deficient Saccharomyces cerevisiae. J. Gen. Microbiol. (1960) 23, 143.

Fazal Ahmad, A. H. Rose and N. K. Garg, Effect of biotin deficiency on the synthesis of nucleic acids and protein by Saccharomyces cerevisiae. J. Gen. Microbiol. (1961) 24

P. O. Hagen and A. H. Rose, A psychrophilic Cryptococcus. (submitted for publication).

XIII. United States Department of Agriculture, Northern Utilization Research and Development Division, Peoria, Illinois. Communicated by Dr. L. J. Wickerham.

The work on new sphingolipides by Dr. Frank Stodola is continuing, and Dr. Hanns Maister is studying their production in pilot-plant operations. The first two papers in this series have been published this year:

Wickerham, L. J., and F. H. Stodola. 1960. Formation of extracellular sphingolipides by microorganisms. I. Tetra-acetylphylosphingosine from Hansenula ciferrii. Journal of Bacteriology, 80 (4) 484-491.

Stodola, F., and L. J. Wickerham. 1960. Formation of extracellular sphingolipides by microorganisms. II. Structural studies on tetraacetylphytosphingosine. Journal of Biological Chemistry, 235 (9): 2584-2585.

We have studied sexuality of Saccharomyces rouxii isolated from miso, the Japanese fermented food made from soybeans. The strains are heterothallic, though the individual mating types self diploidize and sporulate to a small extent for some time after they have been isolated from a bisexual culture. This study is reported in the following paper:

Wickerham, L. J., and K. A. Burton. 1960. Heterothallism in Saccharomyces rouxii. Journal of Bacteriology, 80 (4): 492-495.

On October 12 I took part in a symposium on physiology of fungi in Mexico City, by invitation from the Third National Microbiological Congress. My lecture was entitled "Sexual and Biochemical Changes During the Evolution of the Genus Hansenula." We intend to publish it in a United States journal.

XIV. Laboratory of Kodama Brewing Co., Ltd., Iitagawa, Akita Prefecture, Japan. Communicated by Dr. K. Kodama.

The following is the summary of a lecture at the symposium on "Ecology of Microorganism" held under the auspices of the Institute of Applied Microbiology (University of Tokyo) on November 17 and 18.

Ecological study on the film forming yeasts thriving in "Sake" brewing.

In the brewing process of Japanese "Sake", "Yamahai Shubo", meaning "mother of sake", is one of the classic types of starter and which plays an important role in the cultivation of "Sake" yeast (Saccharomyces Sake). This starter is composed of "Koji" (solid culture of Aspergillus oryzae, employing cooked rice), cooked rice and well water. At the first stage of "Shubo" preparation, a pure culture of yeast is inoculated. This starter, as well as "Koji" is prepared under open conditions which allows other wild flora to grow. In addition, the initial pH of this classic "Shubo" is approximately neutral (unlike that of the modern type of "Shubo", in which lactic acid is regularly added), followed by a sluggish pH-drop due to spontaneously grown lactic acid bacteria. As a result, there must be several kinds of microorganisms which undergo a change during the process of "Shubo" preparation.

The author studied the film forming yeast of the flora mentioned above, which grows at the first stage, preceeding the flora of lactic acid bacteria, the growth of which makes the mash acidic and gives suitable conditions for the growth of "Sake" yeast. Our taxonomic study on this yeast, isolated from 67 samples of "Shubo" (47 breweries), revealed that the Hansenula anomala group (including the var. ciferrii, form. javanica) is widely distributed, rarely accompanied by the Pichia membranaefaciens group.

Next, the growth curve of the H. anomala group during the process of "Shubo" preparation was traced daily by a plate count method,

using a selective medium, which contains K-nitrate as the sole source of nitrogen. This curve gives (as Dr. K. Saito formerly suggested) the following tendency. During the first stage, 8-12 X 10^3 cells per gram originating from "Koji", the cells multiply quickly, at the peak point reaching 3.7-5.0 X 10^7/gr. on the surface of "Shubo" mash (this cell number is rather different from brewery to brewery). After that it quickly decreases, and after about two weeks or so, it almost seems to disappear. Concerning the characteristics of this growth curve, it seems to be due to the various properties of the H. anomala group, listed below:

Strains isolated from "Shubo"

1) As this species does not require vitamins for growth and is rather polyphagic in carbon or nitrogen sources, it can easily grow in the early stage of the preparations when the digestion of the mash has not yet been done thoroughly, and therefore it is not abundant in nutrients.
2) Even at relatively low temperatures, such as 10-15°C at the early stages, they can grow quickly on the surface of the mash.
3) However, as the mash gradually becomes digested and oxygen becomes limited, these aerobic yeasts fail to grow further.

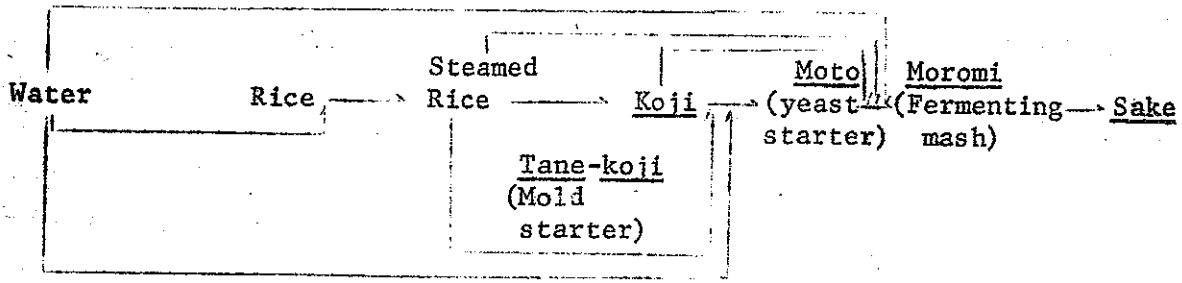
Further ecological studies are conducted in cooperation with Professor T. Uemura and his colleagues of Tohoku University.

XV. Institute of Applied Microbiology, The University of Tokyo, Bunkyo-Ku, Tokyo, Japan. Communicated by Dr. K. Kitahara.

Summary of a lecture presented at the Second Annual Symposium of the Institute of Applied Microbiology, November 17 and 18, 1960.

An Ecological Consideration on Sake-Brewing

Japanese ancestors have established the following process of sake-brewing from rice and water:



If this is considered from the standpoint of modern microbial ecology, some remarkable techniques are found in this classical process.

1) Introduction of Aspergillus oryzae as the saccharifier

Fermentation of sake-mash is started at an initial starch concentration above 30%. Such a process is considered unattainable without Asp. oryzae, a pre-eminent α-amylase producer among molds.

2) Microbial ecosystem: natural pure culture of sake-yeast

In the preparation of moto, many types of microorganisms are introduced from the ingredients as well as from the air and from equipment. And, in the mixture, a succession of populations of predominating organisms normally takes place. 1) Asp. oryzae ceases its action as a living creature, and acts exclusively as an enzyme mixture. 2) Facultative aerobes chiefly Micrococci are the first to propagate and reduce nitrates in water to nitrites which suppress the excessively rapid outgrowth of sake-yeast. 3) Two species of semi-psychrophilic lactic acid bacteria, Leuconostoc mesenteroides and Lactobacillus sake, propagate successively, intercepting the growth of other microorganisms including nitrate-reducers and even foreign lactic acid formers. 4) Under such environmental conditions, sake-yeast begins to grow predominantly. 5) Lactic acid formers die off one after another by synergistic action of lactic acid and alcohol.

Thus, in spite of the process being carried out in an open system, the yeast culture obtained is not only in a pure state but also in an adapted condition. This ecological consideration, however, has led the author to a conclusion that the sake-yeast might be a mixture of several kinds of fermenting yeasts instead of a defined species called Saccharomyces sake.

A bacteriophage attacking Leuconostoc mesenteroides had once been isolated from moto. However, there is nothing to fear about its harmful effect, because the infected cell does not burst at such acidic conditions as exist in moto. However, it permits the intracellular multiplication of phage particles.

3) 'Simultaneous fermentation process' starting with an extraordinary high starch concentration

A key for controlling the fermentation of sake-mash is the harmonization of the yeast fermentation with saccharification by koji-diastase. If they are sufficiently balanced, the initial 'total sugar' in a concentration of over 35% changes into alcohol. This yields a maximal alcohol concentration of 18-20% (by volume), while keeping a very low level of 'free sugar' throughout the fermentation period.

There remains an as yet unsolved problem, that is why such high alcohol concentrations are attainable by yeast fermentation.

4) Pasteurization: a struggle against hiochi-bacilli

Sake is naturally suitable as a culture medium for many kinds of microorganisms if its high alcohol concentration is excepted. Therefore, it is a highly selective medium for lactic acid bacteria with high alcohol tolerance.

It is interesting to see that there are two species of so-called true hiochi-bacilli, Lactob. homohiochii and L. heterohiochii named by the present author (1957). These species cannot grow in any media other than sake. This is because these two species are exacting toward mevalonic acid (hiochic acid, Tamura) which is present in considerable

amounts in sake.

Nevertheless, hiochi-bacilli have long been troublesome to sake-brewers, whose efforts led to a method of heating sake for about 30 minutes at 55-60°C. This discovery is said to be more than 150 years older than Pasteur's similar method.

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

The researches concerning yeasts at our institute are listed below:

1. Microbiological and Technological Studies on Cocoa. Fermentation - R. de Camargo, J. Leme Jr., A. Martinelli. This is the second part of the work being conducted at the "Instituto de Cacau da Bahia", in Uruçuca, Bahia. Strains of S. cerevisiae, Zymomona mobilis, Hansenula anomala and Geotrichum candidum were isolated from cocoa fermentations and tested in experimental fermentations. Apparently none of these organisms gives a better fermentation than those obtained spontaneously. Some other studies were conducted concerning cocoa soil microflora and the presence of Drosophila flies during the fermentation of cocoa. A paper will be published in February 1961, which will cover all the results obtained.

2. Studies on the yield of alcohol during fermentation (black strap molasses and sugar) by using several antibiotics. Luiz G. do Prado. This research was to study the influence of different antibiotics on the yield of alcohol using a pure culture of S. carlsbergensis (IZ-1831).

3. Isolation, identification and utilization of yeasts obtained from black strap molasses in the state of S. Paulo. R. de Camargo. This work shall be initiated next sugar cane season and we hope to obtain knowledge of the important yeasts found in different black strap molasses used as raw material for industrial alcoholic fermentations.

4. Miss R. Nelly Neder and Dr. L. Pinheiro will publish a paper dealing with microorganisms isolated from the air in Piracicaba. This work includes several interesting yeasts isolated

Brief News Items:

1. Professor A. Chaves Batista, Universidade do Recife, Instituto de Micologia, Pernambuco, Brazil, has communicated the following news items:

a) Research on Yeast fungi is carried out at the IMUR on bile, on excrements and on air samples; daily, 200 samples of such materials are collected and received.

b) The epidemiology of Trichosporon cutaneum (De Beurm & Gougerot & Vaucher) Ota, chiefly in young people.

c) A series of IMUR publications has been prepared during the last 2 months covering several aspects of Taxonomy.

d) A course on Ascomycetes, including phylogeny, has been offered by Professor A. Chaves Batista in IMUR. Duration, 2 months.

e) Reginaldo Pessôa Coêlho, zymologist, left IMUR and now is in The People Friendship University of Moscow, U.S.S.R.

f) Dr. Glisthenes Teixeira de Vasconcelos is a new zymologist added to IMUR.

g) Professor Everest S. Beneke and Dr. Alvim L. Rogers were IMUR visitors for a short time.

h) Professor Rolf Singer, from Argentine and his wife spent some weeks working in IMUR on Basidiomycetes.

i) Professor J. A. von Arx, from Baarn, Holland has been here, specially invited by IMUR, during July until the end of October.

j) Dr. George Olah, from Louvain Catholic University was added to IMUR as researcher.

k) Professor Guilermo Casas Rincon from Universidad del Zulia, Maracaibo, Venezuela, spent the month of October here observing yeast research at the IMUR.

l) Dr. Frederich Staib, from the Institute of Hygiene, Wurzburg, Germany is with me during October-December studying human mycotic diseases and particularly levedurosis.

2. Professor C. A. Tobias, Donner Laboratory, University of California at Berkeley, writes the following:

I recently visited Moscow in connection with a conference on Radiation Biology, sponsored by the UNESCO. I found that there are various groups of workers working with yeast cells.

Professor M. N. Meissel, who works at the Institute of Microbiology of the USSR Academy of Sciences, Moscow, has for many years been developing fluorescence microscopy with particular attention to fluoresence of various stains of yeast cells. His recent work, which he presented at Moscow, concerns autofluoresence of cells when they are cooled to extreme cold temperatures. He has demonstrated that there is a near ultra-violet fluoresence from yeast cells which can be excited in the far ultra-violet. Also, he showed that following penetrating irradiations, the fluorescence of the membrane of the mucleus and of the volutin granules is increased and its spectrum is shifted to a longer wave length.

Professor Tarasov at the Biophysics Department of the Moscow University, has been studying the kinetics of primary reactions caused by irradiation and the mechanism of chemical protection. He believes that radiation initiates a chain reaction in cells, probably in their life protein membranes, which leads to impairment of cellular function. This chain reaction depends upon oxygen concentrations, and using yeast cells he has worked on the oxygen effect in cells that are being irradiated. Also, he finds that in a dense suspension of cells radiation forms poisonous products which will augment the killing of other cells.

3. Dr. Huseyin Sahinkaya, Department of Fermentation Technology, College of Agriculture, University of Ankara, Turkey, writes:

The following papers have been published recently:

1) The copper ion requirement of some Turkish wine yeast for growth and fermentation. By Prof. Dr. Arif Akman and Dr. Huseyin Sahinkaya. The Journal of College of Agr., Quarter 2, 1959 (in Turkish).

2) The study on the fermentation of molasses by Turkish wine yeasts. By Prof. Dr. Arif Akman and Dr. Ismet Turker. The Journal of College of Agr., Quarter 3, 1959 (in Turkish).
3) Study of the effects of copper, cadmium and manganese on yeast growth. By Dr. Huseyin Sahinkaya. Wallerstein Lab. Comm., Vol. XXIII, No. 80, April 1960.

4. Dr. H. J. Peppler, Red Star Yeast and Products Company, 221 East Buffalo St., Milwaukee, Wisc., writes:

In the new book, Bakery Technology and Engineering, the chapter concerning YEAST was written by Dr. Henry J. Peppler. Since its release in October, the publishers, the AVI Publishing Co., Westport, Conn., and the editor, Samual A. Matz, report brisk interest.

"Dissolved Oxygen measurement in Yeast Propagations", a paper prepared by Jack A. Strohm and Robert F. Dale, was given by Mr. Dale at the symposium on New Techniques in Fermentation Research and Engineering, Fermentation Subdivision, Division of Agricultural and Food Chemistry, American Chemical Society, 15 September, New York, New York.

Dr. J. A. Thorn is now Manager of Laboratories, Yeast Division.

Dr. A. M. Moustafa, R. L. Mueller and J. A. Strohm, formerly in the Research Department, are employed by Miami Margarine (Cincinnati), Milprint, Inc. (Milwaukee) and Froedtert Malt Corp. (Milwaukee), respectively.

Dr. Gerald Reed discussed "Yeast Fermentation" at a recent dinner meeting of the American Brewing Chemists in Milwaukee. He had talked earlier to the Cereal Chemists of Winnepeg on "Chemistry of Yeast and Yeast Action".

5. From Dr. Leslie R. Hedrick, Illinois Institute of Technology, Dept. of Biology, Chicago 16, Illinois:

In the summer of 1960 we had our second group of high school and college teachers in the NSF sponsored program--Research Participation. In 1960, 6 individuals were selected from about 300 applicants. These teachers studied and did research on yeasts as representatives of the field of microbiology. From reports received, most of these persons are continuing work upon the projects started here at the Institute.

Papers published or in press:

L. R. Hedrick, 1960, Some surface characteristics of Hansenula yeasts as indicated by sedimentation patterns in dilute saline Transactions Ill. State Academy of Science 53, 50-61.

Clare E. Forney and L. R. Hedrick, 1961, Effect of chlortetracycline and Candida krusei on embryonated eggs. Applied Microbiology. In press, for January issue.

6. Dr. H. B. Naylor, Professor of Bacteriology, Cornell University, Ithaca, New York writes:

I would like to report in the Yeast News Letter that Dr. T. R. Thyagarajan of Bangalore, India, is spending a year in our laboratories. He is continuing his studies of the cytology of the yeast nucleus.

7. Dr. Mercedes R. Edwards, State of New York, Department of Health, Albany 1, New York writes:

At the meeting of the American Public Health Association in San Francisco on November 2, Dr. Morris A. Gordon, as a panel member of the symposium on fluorescent antibody, discussed the "Diagnostic Usefulness and Limitations of Fluorescent Antibody Technics as Applied to Pathogenic Yeasts." These methods have been especially useful in separating the species of Candida and demonstrating serologic relationships among Candida, Torulopsis, and other yeasts.

8. University of Pennsylvania, The School of Medicine, Philadelphia 4, Pennsylvania.

Dr. DeLamater is presently (for a six month period) visiting scientist at the Strangeways Research Laboratory and at the School of Botany, Cambridge University, Cambridge, England, studying methods for the electron microscopy of microorganisms.

8. Mr. H. C. Chien, Manager, Food and Feed Yeast Plant, Hsin-Ying, Taiwan, writes:

It is a pleasure to me to inform you that a paper entitled "Free China's Big Protein Project" published in the February issue of Food Engineering might be interesting to subscribers of the Yeast News Letter. I ask the privilege of requesting any of the readers of the Yeast News Letter to contact us about the further development and better use of our yeast for human consumption. Now only one tenth of our yeast goes to human use. We wish to promote this situation intensely.

10. Dr. James A. Barnett, Low Temperature Research Station, Downing Street, Cambridge, England, writes:

1) The suggestions about classifying Saccharomyces cerevisiae that I made in Yeast News Letter (VIII, (2), 32) last November have been published: BARNETT, J. A. & KORNBERG, H. L. (1960). The utilization by yeasts of acids of the tricarboxylic acid cycle. J. Gen. Microbiol. 23, 65.

2) My article, Comparative studies of yeasts - Nature, Lond. 186, 449 (1960) - has evoked some controversy which is going to be published in Nature.

11. Dr. F. W. Beech, Cider and Fruit Juices Section, Research Station, Long Ashton, Bristol England, writes:

For the next two years Mr. J. F. Bowen will be studying the ecology of yeasts in apple orchards and cider factories for a Ph.D.

thesis. He is on sabbatical leave from Summerland Research Centre, Canada, Dept. of Agriculture.

12. Dr. R. C. Artagaveytia-Allende, Department of Enologia-Lab. Micologia, Facultad de Quimica, Montevidco, Uruguay, writes:

In the beginning of this year I was invited to organize a Section of Mycology in the Department of Enology--Faculty of Chemistry. Primarily, studies on yeasts are intended to be carried out in this Section. Professor Marotta works on the ecology of wine yeasts and Miss Bracho and I study the yeasts occurring in water of the river which crosses the city.

A course on yeasts was offered in July and August.

Now some studies on the ecology of the yeasts in Uruguay are being carried out. Some results will be published soon.

Professor Guillermo Casas Rincon, from Maracaibo, Venezuela, spent some months working in this laboratory.

A culture collection is being organized. The respective catalogue will be published and sent to other laboratories.

13. It is planned to hold the usual "Round Table Discussion" on problems related to yeasts during the Annual National Meeting of the Society of American Bacteriologists. The meetings are held in Chicago, Hotel Morrison, April 23-27, 1961. Anyone interested is invited to participate.

Letters to the Editor:

Dear Sir:

I regret to inform you that Dr. Kendo Saito, emeritus professor of Osaka University has passed away on the 14th of October 1960. In his death, microbiology in the Orient has lost one of its most outstanding leaders. On the 6th of this month, his funeral ceremony was solemnly held at "Tsuboi Memorial Hall" of Osaka University under the attendance of many people with whom he was well acquainted, besides his family and many of his followers.

All those who have known Professor Saito will understand how deeply we regret him, both as an excellent microbiologist and as a virtuous professor.

Dr. Kenkichi Kodama

Dear Sir:

A new method of classification of Rhodotorula has been published by us. (J. Gen. Appl. Microbiol. 6, 200-213, 1960) In this system, the genus Rhodotorula includes 12 species and 3 varieties.

In the last Yeast News Letter (Vol. IX, 6, 1960), Dr. F. M. Clark pointed out that a strain of Rhodotorula aurantiaca was used for the

assay of p-aminobenzoic acid. In our test, Rht. aurantiaca, ATCC 9536 used for the assay of p-aminobenzoic acid (W. J. Robbins : Science 100, 85, 1944), assimilated lactose vigorously. From this result, it is concluded that this strain coincided with Rh. lactosa and not with Rh. aurantiaca. The fact is proven amply by its colony color and by the requirement of p-aminobenzoic acid. Very likely Dr. Clark's strain is identical with the above ATCC strain.

A taxonomic discussion should be added also as to the relationship between Rh. glutinis var. infirmo-miniata and Rh. macerans. These two strains are characterized by the production of a starch-like compound in the red Rhodotorulas. According to original description (K. Okunuki, Japanese J. Bot., 5, 285 (1931)), the former strain had assimilated lactose. The property, however, could not be demonstrated by Lodder (1934) and by Lodder and Kreger-van Rij (1952). Recently we have found that both strains required biotin essentially, and the parallelity between the vitamin requirement and other physiological properties is a remarkable feature of the genus. From this, the former seems to be synonymous with the latter and to have lost its lactose assimilability during successive cultivations.

T. Hasegawa

Institute for Fermentation
Juso-Nishino-cho, Higashiyodogawa-ku
Osaka, Japan

Dear Sir:

It is with great pleasure that I have learned, through the Yeast News Letter, of a bond between investigators in the same research area: the yeasts and problems related to this group of fungi.

If I could make a suggestion, it would be to try, by mutual agreement between authors, to systematize the tests used for identification, particularly those used to describe new species. Rather than a multiplication of the tests applied, which might cause a simple variety to be raised to the rank of species, it seems necessary to me to have some standardization, particularly with respect to results of assimilation.

Henri Saëz

Parc Zoologique
Paris XII