

# Y E A S T

A News Letter for Persons Interested in Yeast

November 1963

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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 1964. 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

The Editor extends to the readers of the Yeast News Letter his warmest wishes for a happy and productive new year ahead.

H. J. Phaff

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

The following cultures, for which a description has been published, have been received by the C.B.S.:

Naganishia globosus Goto

S. Goto, J. Ferm. Technol. 41, 459, 1963.

Sacch. vanudenii van der Walt et Nel

J. P. van der Walt and E. E. Nel. Mycopathologia 20, 71, 1963.

Publication: N. J. W. Kreger-van Rij and J. P. van der Walt.  
Ascospores of Endomycopsis selenospora (Nadson et Krassilnikov)  
Dekker. Nature (London) 199, 1012, 1963.

Dr. R. Sceda from Berlin has joined the staff of the C.B.S. (Yeast Division) on September 1, 1963.

II. Centraalbureau voor Schimmelcultures, Julianalaan 67a, Delft, Holland.  
Communicated by Dr. J. Lodder.

Readers of the Yeast News Letter might be interested to hear that plans are being worked out for a new and thoroughly revised edition of "The Yeasts".

During the last decade much work has been done and published on yeast taxonomy, including the descriptions of new genera and species. This has caused that the present edition, which appeared in 1952 and is already out of print, is no longer up to date.

During a conference a few years ago between Mr. M. D. Frank, Director of the North Holland Publishing Co. at Amsterdam and Mrs. N.J.W. Kreger and myself we came to the conclusion that a revision could only be accomplished when several specialists would contribute to it. We discussed this idea with Dr. H. J. Phaff who at that time happened to be in Holland. This all finally led to an agreement between the Publishing Co. and the following scientists, well known for their work on yeast taxonomy, who all will contribute to the new edition:

Mrs. N.J.W. Kreger-van Rij - Delft, Holland

(Endomycopsis, Pichia, Debaryomyces, Schizoblastosporion)

Dr. H. J. Phaff - Davis, Calif., U.S.A.

(Schwanniomycetes, Saccharomycopsis, Saccharomycodes, Hanseniaspora, Nadsonia, Wickerhamia, Sporobolomyces, Bullera, Cryptococcus, Kloeckera, Rhodotorula)

Miss W. Ch. Slooff - Delft, Holland

(Schizosaccharomyces, Lipomyces, Trigonopsis, Pityrosporum)

Dr. N. van Uden - Lisbon, Portugal

(Nematospora, Metschnikowia, Torulopsis, Candida, Trichosporon)

Dr. J. P. van der Walt - Pretoria, S. Africa

(Saccharomyces, Kluyveromyces, Brettanomyces)

Dr. L. J. Wickerham who first declined being a contributor because of his numerous activities, kindly agreed to deal with the genus Hansenula.

The Publishing Co. asked me to act as the Editor,

To make the new edition as complete as possible all people working on yeast taxonomy are kindly requested to send reprints of their papers, and, in case of descriptions of new species, cultures to the contributors of the new edition.

It is not yet possible to make any speculations on the time of appearance of the new edition.

III. Instituto Nacional de Investigaciones Agronomicas, Seccion de Bioquimica, Madrid, Spain. Communicated by Dr. J. Santa Maria.

Since the publication of the last Yeast News Letter the following paper has been published:

"Saccharomyces oleaginosus (Van-Uden & Assis-Lopes) Santa Maria (Basionym. Sacch. italicus Castelli, var. melibiosi, van-Uden & Assis-Lopes, nov. var.)". Bol. Inst. Nac. Inv. Agronomicas (Madrid) 48, 17-32, 1963.

The following articles have been accepted for publication:

1. "New melibiose-utilizing yeasts, isolated from "alpechin". Antonie van Leeuwenhoek. This work includes the description of Torulopsis salmanticensis, nov. spec., Saccharomyces norbensis, nov. spec. and Pichia vini var. melibiosi, nov. var.

2. "On the validity of genus Torulasporea (Lindner) in ascosporegenous yeasts". Bol. Inst. Nac. Inv. Agronomicas (Madrid). A comparative study has been carried out between Torulasporea nilssoni Capriotti and Saccharomyces malacitensis, from which the conclusion has been reached that the need to eliminate the genus Torulasporea must be maintained, and that the two yeasts mentioned should be designated Sacch. nilssoni and Sacch. nilssoni var. malacitensis, respectively.

An article on the utilization of saccharose and maltose by yeasts is now in preparation.

IV. Facultad de Quimica, Montevideo, Uruguay. Communicated by Dr. R. C. Artagaveytia-Allende, (Postal address Salto 1184, Montevideo.)

The genus Rhodotorula, Harrison: some considerations about its actual status. Publicacion No. 382 del Instituto de Micologia de la Universidad de Recife Brasil, 1963. (in Spanish).

The genus Rhodotorula is characterized by the visible presence of red-orange pigments. This is influenced by various factors, such as the temperature and the composition and age of the culture medium, which are principally active.

In this genus great influence of the cultural conditions on the macroscopic appearance of the strains is observed.

Mutations and variations can clearly be observed, as well as the appearance of nonpigmented variants. With the idea to utilize more elements

for the diagnosis the number of tests was increased, producing the description of many new species, which differed from those known until 1932 according to their diversity of assimilation of carbon sources.

Sub-species created and many species which today are considered to belong to other genera, were grouped in the genus Rhodotorula.

The serology in the genus Rhodotorula is at the beginning, and as long as it does not develop further only serological groups will be created.

The multiplication of tests increased the difficulty of identification due to the impossibility to carry out all of them and also because the authors do not follow a common limited scheme. This establishes the obligation to consider the isolated strains as belonging to particular groups.

V. Instituto di Patologia Vegetale e Microbiologia Agraria, Universita di Pisa, Italy. Communicated by O. Verona.

O. Verona e A. Rambelli (1961) - Intorno ad un ceppo di Candida Reukaufii (Gruss) Diddens et Lodder isolato da Fiori di Eucalitto. (A strain of Candida Reukaufii (Gruss) Diddens and Lodder isolated from Eucalyptus flowers.) Atti Ist. Botanico Un. Pavia, Vol. XIX, 77-84.

Verona and Rambelli studied a strain of C. reukaufii and tentatively established its life-cycle. For the dynamic and complex morphology presented by this species, the authors advance doubts about its placement in the genus Candida.

O. Verona e A. Rambelli (1961) - Notizie, ricerche e considerazioni relative "Candida pulcherrima" ed altri lieviti ad analoga fisionomia. (Some notices, researches and considerations upon "Candida pulcherrima" and related yeasts.) Annali Facolta Agraria, Univ. Pisa, Vol. XXII, 91-121.

A comparative study was made of some strains of C. pulcherrima presenting rounded and typical chlamydo-spores, against strains which constantly present oval chlamydo-spores and strains of C. reukaufii.

Such a comparative study indicates that we are dealing with at least two taxa, which are physiologically similar, but are different in their morphometrical aspects.

The authors advance some considerations in the discussion of referring these taxa to the genus Candida. In the authors' opinion, it would be opportune to institute a new genus for C. pulcherrima and C. reukaufii.

This opinion is newly presented by Verona who is particularly interested in the gen. Candida.

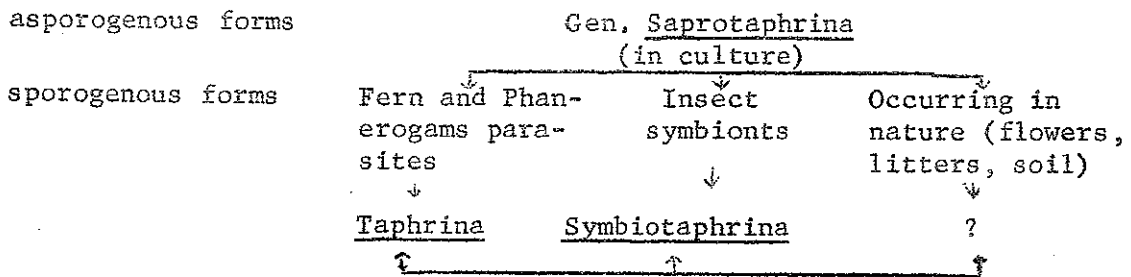
O. Verona and A. Rambelli (1962) - About two isolates labelled Taphrina farlowii Sad. and T. californica Mix. Phytop. Zeitschr., 44. 269-272.

The authors, studying two strains of Taphrina labelled T. farlowii Sad. and T. californica Mix., found that such strains are to be referred to Candida melibiosi Lodder et Kreger-van Rij and Cryptococcus laurentii (Kuff.) Skinner, respectively.

O. Verona e A. Rambelli (1962) - Notizie intorno ai caratteri di alcune specie di Taphrina in fase saprofitaria (Saprotaphrina). (On the characters of some species of Taphrina in saprophytic phase (Saprotaphrina). Annali Facolte Agraria, Univ. Pisa, Vol. XXIII.

Ascospores of Taphrina, when germinating, give rise, as is well known, to a yeast-like vegetation. The authors point out in detail the fundamental morphological and physiological characters of many species.

After observing that also some symbiotic insect-inhabiting strains as well as some strains from common environments present similar characters, the authors advance the proposal to group all of these into a new genus: Saprotaphrina. A diagnosis of the genus is given. Included in it are the asporogenous forms of Taphrina and Symbiotaphrina, as well as the forms which present a taphri- noid habit but which are unknown as sporogenous forms. The genus would there- fore include environmental and biogenic species, the latter living symbiotically on insects (Symbiotaphrina) or parasitically on ferns and phanerogams (Taphrina). In summary:



O. Verona e A. Rambelli (1962) - Presenza di lieviti e di specie lievitiforimi in lettieri di bosco. (On the presence of yeasts and yeast-like organisms in leaf litters of Eucalyptus, etc.) Annali Facolte Agraria, Univ. Pisa, Vol. XXIII, 37-46.

From leaf litters of Eucalyptus, Chestnut and Oak, various yeasts and three species of Saprotaphrina were isolated. The latter are described in detail.

E. Aquarone (1961) - Qualche ricerca su di alcuni lieviti osmofili. (Some researches on certain osmophilic yeasts). Annali Facolte Agraria, Univ. Pisa, Vol. XXII, 195-214.

In the present note some aspects relative to the physiology of the osmophilic yeasts, Saccharomyces mellis and Sacch. elegans, are studied.

The author refers to the pattern of the first phases of the fermentative process in relation to different conditions of osmotic pressure, temperature and pH of the medium; the vitamin requirements of the two species were determined and the thermal death time established; finally the main fermentation products (ethanol, acetic acid, acetaldehyde, glycerol, succinic acid), which are formed under different conditions of osmotic pressure and pH values, were studied.

VI. Institute of Fermentation, Yamanashi University, Kitashin-machi, Kofu, Japan. Communicated by Dr. Goto.

On a new yeast genus Naganishia. Jour. Fermentation Technol. 41, 459-462 (1963). by Shoji Goto.

The author proposes as a new genus a yeast (strain No. 24) which was isolated from Blue cheese. A new yeast genus Naganishia (Naganishia globosus) was established as a member of the Saccharomycetaceae. On cucumber-glucose-agar slants and Kleyn's medium only one oval or round ascospore, which has sparse and irregular warts at times. These ascospores are formed either after conjugation or without conjugation. Nitrate is assimilated and sugar is not fermented. A starch-like compound is produced.

This genus seems to be situated at the intermediate position between Giteromyces or Hansenula and Debaryomyces. This yeast may be regarded as the perfect form of Cryptococcus diffluens.

VII. The Royal College of Science and Technology, Department of Applied Microbiology and Biology, Glasgow, Scotland. Communicated by Dr. Sheena S. Ross and Professor E. O. Morris.

The following is a summary of a part of the work carried out in the above Department and presented in a Ph.D. thesis entitled "A Study of Yeasts of Marine Origin" by Sheena S. Ross:

Altogether from various sources 235 yeast cultures were collected and examined; of these, 189 were from marine fish and 11 from sea-water. The remaining 35 were contributed by other workers. Work was carried out to improve methods for sampling and isolation of these yeasts.

The 213 isolates from fish were found to comprise eight genera - Debaryomyces, Torulopsis, Candida, Rhodotorula, Pichia, Trichosporon, Cryptococcus and Pullularia. Forty-five per cent of these isolates were classified as D. klockeri. T. inconspicua (var) and C. parapsilosis, each comprised 11% respectively.

The 22 isolates from other marine sources belonged to the same genera as the fish isolates and in addition five of the eleven strains isolated from sea-water samples were classified as Metschnikowia zobellii (van Uden and Castelo-branco) nov. comb.

Although most of the isolates agreed closely with the descriptions of the type species as proposed by Lodder and Kreger-van Rij (1952), some differences were obvious and these were studied in the light of possible strain variation within the species.

All the isolates were subjected to further tests other than those proposed by Lodder and Kreger-van Rij (1952). Of these, study of the effects on growth of various concentrations of sodium chloride yielded interesting results.

The maximum concentration of sodium chloride tolerated by the marine isolates was found to be a feature of each strain and there was a correlation regarding halo-tolerance among strains of a particular species isolated from different marine locations. Complex organic nitrogen sources were found to stimulate halo-tolerance in these isolates. As the concentration of NaCl was increased in the growth medium, a prolongation in the yeasts' lag phase was observed but their growth rate remained fairly constant. At the higher concentration of NaCl the lag-phase was prolonged in some cases for up to three weeks after which the exponential phase began suddenly. Further, although the mean generation time was close to that obtained for yeasts grown in lower

concentrations of NaCl, the duration of the exponential phase was optimal in media containing 1.0 - 3.0% NaCl, whereas the other species tested grew best without NaCl.

The occurrence and species distribution of the yeasts isolated from marine fish were studied. The highest incidence of yeast occurrence was obtained from slime and skin samples, the next highest yields were from mouth and gill samples and significantly fewer isolates were obtained from the faeces. Without exception *D. kloackeri* was the predominant species in every geographical location of sampling (East and West Scottish coastal waters and fishing grounds off Iceland), whereas the occurrence of the other species and the proportion of isolates comprising them varied within these areas.

References: Sheena S. Ross and E. O. Morris. "Effect of Sodium Chloride on the Growth of certain Yeasts of Marine Origin". J. Sci. Fd. Agric., 13, 467-475, 1962.

E. O. Morris and S. S. Ross, "Yeasts from Marine Fish". 1st Int. Congr. Food Sci. Technol., London, 1962 (in press).

VIII. Prairie Regional Laboratory, Saskatoon, Sask., Canada. Communicated by Dr. J. F. T. Spencer.

Work in progress:

A survey of the yeasts occurring in the surface waters of Saskatchewan has been started. The waters to be studied include rivers, fresh water lakes, saline lakes and alkaline ponds. An investigation of yeasts contributed to the South Saskatchewan river by the Saskatoon sewage disposal system is also underway.

The structure of the tri- and tetrasaccharides formed by *Sporobolomyces singularis*, a new yeast isolated by H. J. Phaff and Lidia do Carmo-Sousa, have been determined. Further studies of the transglycosidation reactions carried on by this yeast are in progress.

Studies of metabolic pools in *Candida utilis*, grown in continuous culture, are being carried on by P. S. S. Dawson of this laboratory. Mr. Dawson is now preparing a thesis describing this work.

IX. Czechoslovak Academy of Sciences, Chemical Institute of the Slovak Academy of Sciences, Department of Microbiology, Bratislava, Dubravská cesta, Czechoslovakia. Communicated by Dr. Anna Kocková-Kratochvílová.

The department of microbiology of the Chemical Institute of the Slovak Academy of Sciences was founded in 1954. The work of this department is concerned with the following topics:

1. It maintains pure cultures of yeasts, yeast-like microorganisms and some molds. These organisms are isolated from natural and clinical material and identified with the aim of studying their taxonomic, ecologic and biochemical properties.

2. It studies the biochemistry and enzymology of yeasts, biochemical mechanisms of cells as well as cell-free extracts, activity of enzymes and their localization in cells.

3. It cultivates yeasts and yeast-like microorganisms in a greater quantity and carries out chemical analyses of fermenting liquids and biomass. It cultivates these microorganisms by different ways: mixing, aeration, shaking and by continuous culture.

4. It is engaged in serologic research of yeasts; it studies the spectra of antigens, verifies their activity and chemical nature. It tests the toxicity and pathogenicity of strains.

These methods found an application for biochemical and taxonomic research. In the last two years we were engaged mainly in research of the genus Candida Berkhout. A fundamental standardization of Candida albicans, C. tropicalis, C. pulcherrima was carried out. Phylogenetic affinity of yeasts of the "II. fermentation type", which ferments maltose and sucrose, of the genus Saccharomyces, Candida and Torulopsis was studied. Other fermentation types are: I. maltose +, sucrose -; II. maltose +, sucrose +; III. maltose -, sucrose +; IV. maltose -, sucrose -.

Since the year 1962 the following papers have been published or submitted for publication:

Kockova-Kratochvilova A., Sandula J., Hronská L. The genus Candida Berkhout. I. Basic Typization of Candida albicans (Robin) Berkhout. Folia microbiologica 8, 109-116, 1963.

Sandula J., Kockova-Kratochvilova A., Zamecnikova M. The genus Candida Berkhout. II. Pathogenicity of Candida albicans (Robin) Berkhout. Folia microbiologica 8, in press, 1963.

Kockova-Kratochvilova A., Sandula J., Vojtkova-Lepsikova A. The genus Candida Berkhout. III. Intermediate forms between the first and second fermentation type. Folia microbiologica 8, in press, 1963.

Kockova-Kratochvilova A., Vojtkova-Lepsikova A. The genus Candida Berkhout. IV. Importance of molybdate test for identifying the species of the Candida genus. Cs. epidemiologie, mikrobiologie, imunologie 12, 184-187, 1963.

Sandula J., Kockova-Kratochvilova A., Zamecnikova M. The genus Candida Berkhout. VI. To the question of the pathogenicity of strains of the Candida albicans. Biologia, in press.

Vojtkova-Lepsikova A., Kockova-Kratochvilova A., Fischerova M. The genus Candida Berkhout. VII. Formation of organic acids during the metabolism of glucose by the species of the genus Candida. In press.

Pilat A., Kockova-Kratochvilova A. Carposomata agarici xanthodermi Gen. fungo saccharomycetiformi Candida humicola (Daszewska) Diddens et Lodder infecta et deformata. Ceska mykologie 16, 83, 1962.

Kockova-Kratochvilova A., Hronská L., Kalesova L. To the question of yeast ecology. Yeasts and yeast-like microorganisms on the plants of Southern Slovakia. Ceska mykologie, in press.

Kockova-Kratochvilova A. Die Typisierung untergäriger Brauereihefe. Brauwissenschaft 15, 390, 1962.



Kockova-Kratochvilova A., Sandula J. Serologic method for evidence of cultural and wild yeasts. Kvasny prumysl 8, in press, 1963.

X. Biological Institute, College of General Education, Hiroshima University, Hiroshima City, Japan. Communicated by Dr. Minoru Yoneyama.

1. The 13th regular meeting of the seminar of yeast studies was held in Osaka, Japan on Oct. 16, 1963. This was organized by Drs. Hideo Takada and Naohiko Yanagishima, Professors of Osaka City University, Sugimoto-cho, Sumiyoshi-ku, Osaka. The titles of some of the speeches, which were given at this meeting, are as follows:

1. Takeshi Yamamoto: Heredity and physiology of a strontium resistant variant yeast.
2. Tetsuro Murayama: Metabolism of a copper resistant variant of yeast.
3. Shinichi Okuda: Sulfur metabolism of yeast.
4. Minoru Yoneyama: The yeasts associated with Drosophila flies.
5. Tosiaki Takahashi: Genetic study on the sex of the genus Saccharomyces.
6. Kaoru Banno: Some aspects of the mycelial stage as exhibited by a certain species of Rhodotorula.
7. Tatsuo Yamamoto and Yasuhiro Kariya: Permeability of the cell wall of some yeasts.

2. During the past year, I have obtained some information on yeasts associated with Drosophila flies, such as D. melanogaster, D. immigrans, D. brachynephros, D. trivittata, D. lutea, in Japan. I am especially interested in those from D. melanogaster, a so-called garbage species since this fly is cosmopolitan and the yeast-flora of this insect was characteristic of the "spoilage flora" of spoiled foodstuff or garbage. The yeasts isolated from the crops of D. melanogaster collected from summer to autumn in 1962 in Hiroshima, Japan were, for example, as follows:

Hansenula anomala, Debaryomyces kloeckeri, Pichia sp. Saccharomyces cerevisiae, Candida krusei, Candida solani, Candida guilliermondii, Candida parapsilosis, Candida intermedia, Kloeckera apiculata, Torulopsis famata, Torulopsis dattila, Torulopsis glabrata, Torulopsis sake.

XI. Illinois Institute of Technology, Biology Department, Chicago 16, Illinois. Communicated by Dr. L. R. Hedrick.

Two articles are in press in the Journal of Bacteriology.

1. Hedrick, L. R. and Feren, J. C. Dec. 1963. J. Bact. "Effect of Cations upon Hydrophobicity of Yeasts grown on Amino Acids"

ABSTRACT

Cells of four species of Hansenula, namely H. holstii Y2154, H. subpelliculosa Y1683 and Y1542, and H. anomala Y365 were cultured for three days in a glucose-salts medium with one amino acid or  $(\text{NH}_4)_2\text{SO}_4$  as a N-source. The L-amino acids used were: glutamic acid, valine, proline, lysine, histidine, phenylalanine, tyrosine, and tryptophan. After growth, the cells were washed and added to small tubes which contained varying concentrations of chlorides

of the cations  $Cs^+$ ,  $K^+$ ,  $Na^+$ , and  $Al^{+++}$ . The concentration of salt in ionic strength required for sedimenting yeast cells to form a fixed pellet represents an index of hydrophobicity of the cells, i.e. the smaller the ionic strength to accomplish sedimentation, the greater the hydrophobicity.

All species grown in  $(NH_4)_2SO_4$  had a high degree of hydrophobicity. Cells of H. holstii and H. subpelliculosa Y1683 were very hydrophilic when cultured in the three aromatic amino acids as N-source and in proline as a carbon and a nitrogen source, cells of the latter species were much more hydrophobic if grown with proline as a nitrogen source. Cells of H. holstii cultured on L-glutamic acid as a nitrogen source were much more hydrophobic than those grown on proline as either the N or C-source. Cells of all species grown in L-lysine were more hydrophobic than cells cultured on other amino acids, tested. Hansenula subpelliculosa Y1542 cells were somewhat more hydrophilic than those of H. anomala Y365 but both were much more hydrophobic than the other two yeasts studied.

The concentration of cations required to detect an identical degree of hydrophobicity of cells decreased in the order Cs, Li, K, Na and Al. This order corresponds to the compressibility method for estimating the water of hydration of the cations.

2. Nero, L. C., Mae-Goodwin Tarver and L. R. Hedrick. Jan. 1964. J. Bacteriol. "Growth of Acanthamoeba castellani with the yeast Torulopsis famata".

#### ABSTRACT

Acanthamoeba castellani and Torulopsis famata cells were cultured together for ten days at three different temperatures, 18°C, 25°C, and 32°C. In the 18°C and 25°C series, the yeast population reached a peak within three to five days and then declined for the next five days. This decrease in yeast population corresponded with an increase in the development of amoebae, first as vegetative cells which later transformed themselves into cysts. The changes in population were followed by counts in a haemocytometer. The vegetative amoeba population seldom exceeded  $4 \times 10^7$  cells per bottle-culture, but the encysted population approached  $16 \times 10^7$  cysts per bottle-culture within 10 days of incubation. The amoebae actively ingested the yeasts and used these cells as their principal energy source. At 18°C each amoeba consumed 70 yeast cells per day; at 15°C each amoeba ingested 35 yeast cells per day. In the 32°C series, both the yeast and the amoebae demonstrated slow growth rates. The development of cysts corresponded with the growth of the yeast population without the rapid increase in yeast population prior to the growth of the amoebae cells.

3. Marjorie Soyugenc, Philip Dupont and Richard Ambrosini are identifying yeasts isolated from different fresh water environments - from the Great Lakes, small lakes, rivers and ponds.

XII. Department of Bacteriology, University of Wisconsin, Madison, Wisconsin.  
Communicated by Dr. H. O. Halvorson.

Dr. Roger Bretthauer and Dr. Leon Marcus have recently observed that short polynucleotide fragments serve as synthetic messenger RNA for amino acid incorporation in cell free systems in yeast. The incorporation of phenylalanine in response to poly U<sub>7</sub> - U<sub>25</sub> increases in proportion to chain length as

expected on the basis of the triplet code. Experiments are now in progress to measure the length of the peptide chain in response to synthetic polynucleotides (poly A and poly U) of known chain length.

Dr. Gorman has just completed a study of the purification, characterization and induction specificity of isomaltase. Isomaltase is distinct from  $\alpha$ -glucosidase;  $\alpha$ -Methyl glucoside specifically induces isomaltase and maltose specifically induces  $\alpha$ -glucosidase.  $MG_1$  and  $MG_3$  are regulatory genes for isomaltase. Both  $MG$  and  $mg$  forms of the gene were found to be active and thus provide both positive and negative types of control. His dissertation was "A Study of the Specificity and Genetic Control of  $\alpha$ -Glucosidases in Yeast." He is currently on a post-doctoral fellowship with Dr. P. Slonimski, Gif-sur-Yvette.

The following have joined our group:

Dr. Claudio Heredia from the Institute for Advanced Scientific Research in Madrid.

Dr. Y. Kobayashi from The University of Tokyo.

Dr. Y. Nakao from the Takeda Chemical Industries, Osaka, Japan.

Mr. Patric Tauro from Mysore City, India.

#### Recent Publications:

THE EFFECT OF POLY U SIZE ON THE INCORPORATION OF PHENYLALANINE IN THE CELL-FREE YEAST SYSTEM by L. Marcus, R. K. Bretthauer, R. M. Bock, and H. O. Halvorson. National Academy of Sciences, Vol. 50 pp. 782-789. Oct. 1963.

THE EFFECT OF GENE DOSAGE ON THE LEVEL OF  $\alpha$ -GLUCOSIDASE IN YEAST by F. Rudert and H. O. Halvorson. Bull. Res. Council. of Israel, Vol. 11A4, 1963.

GENE-CONTROLLED FACILITATED DIFFUSION AND ACTIVE TRANSPORT OF  $\alpha$ -THIOETHYLGLUCOPYRANOSIDE IN SACCHAROMYCES CEREVISIAE by Hirosuke Okada and H. O. Halvorson. Biochim. Biophys. Acta, 71 (1963) 229-232.

AMINO ACID INCORPORATION INTO PROTEIN BY CELL-FREE EXTRACTS OF YEAST by R. K. Bretthauer, L. Marcus, J. Chaloupka, H. O. Halvorson and R. M. Bock. Biochemistry (1963) 2, 1079.

THE ROLE OF AN  $\alpha$ -METHYL GLUCOSIDE PERMEASE IN THE INDUCED SYNTHESIS OF ISOMALTASE by H. O. Halvorson, H. Okada and J. Gorman. Soc. Gen. Physiol. Symp. on Cellular Functions of Membrane Transport (in press).

UPTAKE OF  $\alpha$ -THIOETHYL-D-GLUCOPYRANOSIDE BY SACCHAROMYCES CEREVISIAE by Hirosuke Okada and H. O. Halvorson. BBA (in press).

UPTAKE OF  $\alpha$ -THIOETHYL-D-GLUCOPYRANOSIDE BY SACCHAROMYCES CEREVISIAE, II General Characteristics of an Active Transport System by Hirosuke Okada and H. O. Halvorson. BBA (in press).

COMPARISON OF THE ACTIVE TRANSPORT SYSTEMS FOR  $\alpha$ -THIOETHYL-D-GLUCOPYRANOSIDE AND MALTOSE IN SACCHAROMYCES CEREVISIAE by Hirosuke Okada and H. O. Halvorson. J. Bact. (in press).

XIII. Rutgers - The State University, Department of Bacteriology, New Brunswick, New Jersey. Communicated by Dr. W. W. Umbreit.

Drs. E. Hebeka and M. Solotorovsky (Dept. of Bacteriology, Rutgers University, New Brunswick, N. J.) have studied strains of Candida albicans for development of resistance to candidin, amphotericin B and nystatin in liquid medium and by gradient agar plating. After 25 transfers, resistance developed toward candidin and amphotericin B but not toward nystatin. Where development of resistance was observed, the strains lost virulence and the ability to reduce bismuth sulfite. When resistance was developed to candidin, it was accompanied by resistance to amphotericin B, but not to nystatin, fungimycin, griseofulvin or eulicin. Development of resistance to amphotericin B was accompanied by resistance to candidin but not to the other test antibiotics. Both amphotericin B and candidin-resistant strains were more sensitive to eulicin than their parent strains.

Metabolic studies were performed with strains rendered resistant to candidin. Resistant strains showed a reduced R.Q. with glucose, a reduced  $QO_2$  with glucose, citrate, pyruvate, oxalacetate, cysteine and cystathionine, and a rise in  $QO_2$  with glycine, serine, threonine, methionine, homocysteine, valine, leucine and aspartic acid.

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

Present research projects with Schizosaccharomyces pombe are:

(1) The analysis of the inhibitory effect of L-methionine in the plating medium upon expression of spontaneous and induced adenine-independent reverse mutations. The degree of this inhibition depends both on the particular mutant and the particular mutagen used, and can lead to apparent cases of an influence of a met<sup>-</sup> marker on reverse mutability of an adn<sup>-</sup> strain. Apparent mutagen specificity observed in adn<sup>-</sup> met<sup>-</sup> strains can also result from this methionine effect. The effect seems to be specific for L-methionine and is not abolished simply by allowing increased residual divisions of adn<sup>-</sup> cells on the minimal medium plates upon which reversions are scored.

(2) Genetic analysis of spontaneous and induced revertants of adenine auxotrophs to detect suppressor mutations. Results obtained indicate that the same mutant may with one mutagen give revertants mainly of an unlinked suppressor type, whereas with a second mutagen the revertants obtained contain no detectable extra-genic suppressor.

(3) The problem of delayed mutation is being studied in a red-white sectoring colony system involving forward mutations at adenine loci.

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Dr. Nicola Loprieno from Pisa is spending six months in Edinburgh working mainly on reverse mutations of a methionine-4 auxotroph, with HNO<sub>2</sub>, U.V., di-epoxybutane and other mutagens.

Mr. M. Anwar Nasim from Pakistan is here on Colombo Plan grant and is working with the red-white mosaic colony system.

## Publications

- Clarke, C. H. and Bond, G. Heredity 17 (4), 602, 1962 (abstract).
- Clarke, C. H. Z.f. Vererbungsl. 93 (3), 435-440, 1962.
- Clarke, C. H. J. gen. Microbiol. 31 (3), 353-363, 1963.
- Clarke, C. H. Proc. XI Int. Cong. Gen. (The Hague) 1963. Vol. 1 (abstracts) 5.16, p. 61.

## XV. 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:

1. Hirano, Tadashi and Lindegren, Carl C. Electron microscopy of mitochondrial changes in Saccharomyces. J. Ultrastructure Research 8: 322 (1963).
2. Lindegren, Carl C. The receptor-hypothesis of induction of gene-controlled adaptive enzymes. Journal of Theoretical Biology 5: 192-210 (1963).
3. Ouchi, Seiji and Lindegren, Carl C. Genic interaction in Saccharomyces. Canadian Journal of Genetics and Cytology 5: 257 (1963).
4. Lindegren, Carl C. Repressors, Cryptic "Enzymes" and Structural Genes, Nature 199: 720 (1963).
5. Lindegren, Carl C. The nucleoprotein layer of the yeast cell. Nature 198: 1325-1326 (1963).
6. Hwang, Yuh Lin, Lindegren, Gertrude and Lindegren, Carl C. Mapping the Eleventh Centromere in Saccharomyces. Canadian Journal of Genetics and Cytology 5: 290-298 (1963).
7. Lindegren, Carl C. The Lamarckian Proteins. Nature 198: 1224 (1963).
8. Lindegren, Carl C. The flaccid cell wall of Saccharomyces. Canadian Journal of Genetics and Cytology 5: 254-56 (1963).

## XVI. Institut für Gärungsgewerbe, Mikrobiologische Abteilung, Seestrasse 13, 1 Berlin 65 (West). Communicated by Dr. Siegfried Windisch.

Investigations of the yeast flora of beers was concluded for the time being. Candida parapsilosis was the only yeast which was not suppressed during the main fermentation of beer. A study was made of the viable yeast count in beer and on the composition of the yeast flora as influenced by the following factors: 1) beer filtration, 2) the type of container (wood or metal with or without artificial coating), 3) treatment of the bottles (sterilization with or without SO<sub>2</sub>-treatment). A relationship was found between the number of Saccharomyces carlsbergensis and other Saccharomyces species on the one hand and the film yeasts (mainly Pichia membranaefaciens) and all other

yeasts on the other hand. It was possible to arrange the Saccharomyces species found in a series characterized by a loss in fermentation and assimilation properties. This led to a hypothesis regarding the relationship between the Saccharomyces species, which was tested experimentally. (S. Windish, Monatsschrift für Brauerei 15, 203, 1962.)

Dr. H. Gutz from our laboratory has finished an inaugural dissertation on the genetic fine structure of the  $ad_7$  and  $ad_6$  loci of Schizosaccharomyces pombe. The work was partly done in collaboration with Prof. Leupold (Bern, Switzerland). Abstracts of the results have been given at the 11th International Congress of Genetics in Scheveningen this year (H. Gutz: Proceed. 11th Internat. Congr. Genetics, Vol. I, 7; U. Leupold a. H. Gutz: *ibid*, in press). Publications of the full results are in preparation.

Gutz has analyzed the intragenic distribution of 90  $ad_7$  and 216  $ad_6$  mutations induced with nitrous acid. In addition some X-rays induced mutations have been examined. The nitrous acid mutations show an intragenic distribution which is quite different from that found by Leupold for mutations induced with ultraviolet light. Especially should be mentioned that in both loci the nitrous acid induced mutations show pronounced hot spots. One nitrous acid- and one X-ray-induced mutant ( $ad_6$ ) give considerable enhanced recombination frequencies at the intragenic level.

The  $ad_6$  mutants show the phenomenon of allelic complementation; in the  $ad_7$  gene allelic complementation does not occur. The complementation pattern of the  $ad_6$  mutants is very complex and cannot be represented either in a linear or in a circular complementation map.

In the area of yeast genetics the following studies were concluded:

For the fermentation of palatinose the presence of the dominant alleles of the genes  $P_1$  and  $P_2$  are necessary. The gene  $P_1$  directs the formation of a specific palatinase, the gene  $P_2$  controls a permeability factor. The enzyme specifically responsible for splitting palatinose is not identical with  $\alpha$ -glucosidase. (C. C. Emeis, Komplementäre Genwirkung bei der Vergärung von Palatinose durch Hefen der Gattung Saccharomyces, Zeitschrift für Naturforschung 17b, 633, 1962.)

During the investigation of yeasts belonging to Saccharomyces six strains were isolated which showed the same superattenuation in wort as Saccharomyces diastaticus, previously isolated by Gilliland. Experiments with these yeasts showed that starch degradation observed in plate cultures was not directly related with the property of superattenuation. Starch degradation can be observed for many yeasts belonging to Saccharomyces. For this reason it does not seem logical to use the property of starch degradation as a basis for the description of a new species (S. diastaticus). The relatively frequent occurrence of superattenuative yeasts in spoiled beers and their property to rapidly ferment the higher carbohydrates in wort, indicated these yeasts are typical spoilage organisms. (C. C. Emeis, Übervergärende Hefen, Monatsschrift für Brauerei 16, 33, 1963.)

A series of yeasts were prepared, members of which differed only from each other in their degree of ploidy. The series ranged from homozygotic haploid to hexaploid yeasts and the series was used for quantitative fermentation experiments in wort. It was found that yeasts which are only distinguished by their degree of ploidy showed no significant differences in

the fermentation. Heterozygotic triploids and tetraploids, which were obtained by crossing of members of two different homozygotic series, on the other hand, showed a significant increase in the fermentation velocity as compared to the homozygotic and the diploid, penta- and hexaploid heterozygotic strains. (C. C. Emeis, Kulturversuche mit polyploiden Hefen, EBC-Proceedings, in press).

During an investigation of bottom yeasts it was observed that many yeast strains have the tendency to form top fermenting cells, which after isolation can be cultured as stable top fermenting cultures. Mutation is assumed to be the cause of this change. A change in raffinose fermentation is not connected with the change from bottom to top fermentation. The top fermenting mutants still ferment raffinose completely. (C. C. Emeis, Gärverhalten untergäriger Bierhefen, Monatsschrift für Brauerei, in press).

A still unsolved problem is the complete genetic analysis of technical beer yeasts. As a preliminary to this a mass crossing technique was applied involving lithium chloride sensitive spores of homothallic strains with lithium chloride resistant haploids of heterothallic strains. From these hybrids haploid clones were isolated with defined maltose genes, which were used for the determination of maltose genes of bottom fermenting yeasts. With this method strains of S. carlsbergensis could be successfully investigated. (H. Oeser, Genetische Untersuchungen Über das Paarungstypverhalten bei Saccharomyces und die Maltose-Gene einiger unter-gäriger Bierhefen. Archiv für Mikrobiologie 44, 47, 1962).

During further investigations on the genetic analysis of bottom fermenting beer yeasts, ten strains were studied in detail for sporulation, conjugation, fermentation properties, and vitamin requirements. The results gave indications that the strains were pentaploid or hexaploid. Among others it could be demonstrated that of the ten yeasts seven were heterozygotic to the fermentation of maltose, four for the fermentation of sucrose, three for the fermentation of galactose, and nine for the genetic factors controlling vitamin requirements. (H. Oeser und S. Windisch, Genetische Untersuchungen an untergärigen Bierhefen, Monatschrift für Brauerei 16, 137, 1963).

The investigations on maltose and glucose fermentation by homozygotic yeast strains with different genom numbers by Dr. R. Scheda led to new views on the relation between fermentation intensity on the one hand and ploidy on the other hand, as well as the type and number of the maltose genes involved. (Archiv für Mikrobiologie, 45, 65, 1963).

XVII. The Research Laboratories of Kirin Brewery Co., Ltd., 17 Namamugi, Tsurumi-ku, Yokohama, Japan. Communicated by Dr. Yasuo Umeda.

Dr. Yasushi Yamamoto and Mr. Takashi Inoue in our laboratories isolated two strains of yeast, one belonging to S. cerevisiae and the other to S. carlsbergensis. These strains are of interest in their inability to ferment maltotriose and in their characteristic pattern observed in the course of fermentation of wort, that is, both strains of yeast flocculate in earlier stage than those of brewery culture yeast. Some observations on these strains of yeast have progressed and the following articles have been published in "The Report of the Research Laboratories of Kirin Brewery Co., Ltd."

Yamamoto, Y., Inoue, T. Studies of Poor-attenuative Yeast  
Part I. Permeation of maltotriose through the cell wall of the poor-attenuative yeast. Rep. Research Labs. Kirin Brewery, No. 4, 49 (1961).

Part II. Methods for the detection of poor-attenuative yeast cells in the presence of high-attenuative yeast cells and some observation on the growth conditions. Rep. Research Labs. Kirin Brewery, No. 5, 11 (1962).

Part III. The poor-attenuative yeast in mixed fermentation system with the high attenuative yeast. Rep. Research Labs. Kirin Brewery, No. 5, 17 (1962).

XVIII. Laboratorio de Fermentaciones y Enologia, Facultad de Quimica, Montevideo, Uruguay. Communicated by Professor C. R. Cano Marotta.

In the Zymology Section, Mrs. D. Bracho de Kalamar and Prof. C. R. Cano Marotta continue the study of "The yeasts of the wine fermentation in Uruguay". They have recently finished a study in a second zone: II- "El Rincon de las Gallinas".

This zone is situated between two large rivers, near their junction. This zone has been recently incorporated in the viticulture.

It keeps the largest plantings of hybrid vines in our country.

Yeasts isolated from musts of grapes cultivated in the lower and higher regions of the vineyard were classified. Also yeasts isolated from musts of grapes, which had a little dried, were classified.

Differences between the yeast floras of these different musts and respective wines were not observed.

Eighty-six strains were isolated before, during and after the main fermentation.

In the absence of SO<sub>2</sub> the fermentation always begins by Kloeckera apiculata, capable of producing low percentages of alcohol (less than 4% by volume). The fermentation is always completed by Sacch. cerevisiae var. ellipsoideus capable of producing from 12.7% to 16.5% by volume. In the main fermentation Sacch. carlsbergensis capable of producing from 14% to 15% alcohol by volume, were isolated in 11% of the cases.

This is a yeast flora which shows little variation in wine fermentation.

#### Publications

Mrs. D. Bracho de Kalamar. "A note on the presence of dipicolinic acid (2,6 pyridinedicarboxylic acid) in yeast spores". C.O.R. Vol. 1, No. 2, pg. 121 (1962).

"In some experiments we found dipicolinic acid in the cells that were cultivated with glutamic acid (presporulation medium). It was not found in vegetative cells of the same yeast. Although the results obtained up to now are rather irregular the main facts, which have so far not been described in the literature, are given here."

C. R. Cano-Marotta and D. Bracho de Kalamar. "I lieviti della fermentazione vinaria in Uruguay". I - Zona "El Colorado". An. Accad. Italiana della Vite e del Vino, Vol XIV (1962). (cf. Yeast News Letter, Vol. XI, No. 1, 3 (1962)).



XIX. Brief News Items.

1. Dr. H. E. Snyder, Iowa State University, Department of Dairy and Food Industry, Ames, Iowa, writes:

I should mention that we have Lothar E. Leistner working with us now. Dr. Leistner was formerly at the German Institute for Meat Research at Kulmbach, and he is now studying the microbiology of cured meats, particularly that of ham and sausage. In the course of this work, he has isolated quite a few Debaromyces sp. and he is interested in communicating with any other persons who might be working on the microbiology of cured meat.

2. Dr. Edward D. DeLamater writes: I would like to inform you of my transfer to the New York Medical College as Chairman of the Department of Microbiology. Our address is 5th Avenue at 106th Street, New York 29, N. Y.

Dr. Edward Steers of the University of Pennsylvania is joining the Department as Professor of Bacteriology. Dr. Patrick Echlin will return from Cambridge University to join the Department in the future and Dr. Eileen Pike will receive her degree from Columbia University in Parasitology shortly and will join the Department in that capacity.

Other members of the Department have yet to be selected.

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

I am going to visit the U.S.A. September 17, 1963, for studies on the autoradiography at the electron microscope level, which will be carried out for about one year under the direction of Prof. Keith R. Porter in The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge 38, Massachusetts.

I am hoping to meet as many persons interested in yeast as is possible during my stay in the U.S.A.

4. Dr. R. C. Hatfield of the California Polytechnic Institute is spending a year's leave of absence with the World Health Organization under a National Institutes of Health grant. He is located in Buenos Aires.

5. Dr. P. Bréchet, Institut Pasteur, 25 Rue du Docteur Roux, Paris reported the following two publications:

P. Bréchet et al. Comptes rendus, 252, 215-217, 1961.

In this note the authors report the decrease in rate of survival and the degree of preservation of the fermentative and respiratory systems which they have observed in the course of storage of a bakers yeast which had been lyophilized or dried in contact with bentonite.

Another publication from our laboratory is: "Identification des levures d'un moût de Beaujolais au cours de Sa fermentation, by P. Bréchet, J. Chauvet, and H. Girard. Ann. Technol. Agric. 11, 235-244 (1962).

6. The titles and speakers of the Seminar of Yeast-cell Studies in Tokyo during this year (1963) were as follows:

- 1) Yoneta, Y.: On the nucleus of the yeast cell.
- 2) Yuasa, A.: Cytology of yeast cells.
- 3) Tsubaki, K.: Phylogeny of imperfect yeasts.
- 4) Kuraishi, K.: Physiology of yeast cells in the case of nutrient deficiency.
- 5) Tanaka, K.: Electron-microscopic studies on several fungi.
- 6) Miura, J.: Irregular segregation in Saccharomyces.
- 7) Imabori, K.: Protein synthesis by noncellular forms of yeasts.
- 8) Sugaya, S.: On the origin of Sake-yeast.
- 9) Mizushima, S.: Quantitative analysis of the glycolytic enzyme system of lactic acid bacteria.
- 10) Sugimura, T.: Co-enzymes of yeasts.

This year two symposia on yeasts were held in Okayama (Oct. 13, 1963) and Osaka (Oct. 16, 1963) and many persons discussed yeast problems vigorously.

Akira Yuasa  
Department of Biology  
College of General Education  
University of Tokyo  
Komaba, Meguroku  
Tokyo

7. The following three papers were received from Dr. L. R. Batra, Department of Botany, University of Kansas, Lawrence, Kansas:

Batra, Lekh R. and Helene Francke-Grosmann. Contributions to our knowledge of ambrosia fungi. I. Ascoidea hylecoeti sp. nov. (Ascomycetes). Am. J. Bot. 48, 453-456 (1961).

Batra, Lekh R. Habitat and nutrition of Dipodascus and Cephaloascus. Mycologia 55, 508-520 (1963).

Batra, Lekh R. Contributions to our knowledge of ambrosia fungi. II. Endomycopsis fasciculata nom. nov. (ascomycetes). Am. J. Bot. 50, 481-487 (1963).

8. The following series of five papers were received from Dr. Sumio Suehiro, Fisheries Laboratory of Kyushu University, Tsuyazaki, near Fukuoka, Japan:

Suehiro, Sumio. Studies on the yeasts developing in putrefied marine algae. Science Bull. Fac. of Agric., Kyushu Univ., Vol. 17, No. 4 (1960).

Suehiro, Sumio. Studies on the marine yeasts. II. Yeasts isolated from Thalassiosira subtilis (marine Diatom) decayed in flasks. Science Bull. Fac. of Agric., Kyushu Univ., Vol 20, No. 1 (1962).

Suehiro, Sumio. Studies on the marine yeasts. III. Yeasts isolated from the mud of tideland. Science Bull. Fac. of Agric., Kyushu Univ., Vol. 20, No. 2 (1963).

Suehiro, S., Y. Tomiyasu and O. Tanaka. Studies on the marine yeasts. IV. Yeasts isolated from marine plankton. J. Fac. Agric., Kyushu Univ., Vol. 12, No. 3 (1962).

Suehiro, S. and Y. Tomiyasu. Studies on the marine yeasts. V. Yeasts isolated from seaweeds. J. Fac. Agric., Kyushu Univ., Vol. 12, No. 3 (1962).

9. From the Department of Food Science and Technology, Univ. of Calif., Davis:

Two papers were published since the last issue of the Yeast News Letter:

M. J. Lewis and H. J. Phaff. Release of nitrogenous substances by brewers yeast. 2. Effect of environmental conditions. Proc. Am. Soc. Brewing Chemists 1963, pg. 114-123.

H. J. Phaff. Cell wall of yeasts. Annual Rev. Microbiology 17, 15-30, 1963.

10. From the Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Kyoto, Japan:

The following two papers were recently published:

Yoshio Tani (co-authors M. Shimoide, K. Sumino, and S. Fukui).  
Physiological and Biochemical Studies on Saccharomyces sake.  
I. Influences of culture conditions on coagulation of Sacch. sake occurring in media containing lactic acid; II. Vitamin requirements of S. sake in a medium containing lactic acid, especially the interrelationship between the action of lactic acid and inositol.  
Jour. Fermentation Technology 41, 445-450 and 523-530 (1963).