### YEAST 8

### A News Letter for Persons Interested in Yeast

May 1959

Volume VIII, Number 1

### Editor

Herman J. Phaff, University of California, Davis, California

## Associate Editor

Leslie R. Hedrick, Illinois Institute of Technology, Chicago, Illinois

### Associate Editor

F. M. Clark, University of Illinois, Urbana, Illinois

### Associate Editor

Cecil G. Dunn, Massachusetts Institute of Technology, Cambridge, Massachusetts

#### \*\*\*\*\*

<u>Name</u>	Page
N.J.W. Kreger-van Rij, Delft, Holland	2
O. Verona, & A. Montemartini, Pisa, Italy	3
Wm. Bridge Cooke, Cincinnati, Ohio	4
T. Hasegawa, Osaka, Japan	- 5
John M. Carpenter, Lexington, Kentucky	5
Samuel P. Meyers, Miami 49, Florida	6
T. R. Manney, Berkeley 4, California	6
Carl C. Lindegren, Carbondale, Illinois	7
Thomas D. Brock, Cleveland 6, Ohio	7
Marilyn Kvetkas & L. R. Hedrick, Illinois Institute of Technology	8
K. Kodama, Akita Prefecture, Japan	8
H. Katznelson, Ottawa, Canada	10
F. M. Clark, Urbana, Illinois	10
L. J. Wickerham, Peoria, Illinois	10
H. O. Halvorson, Madison 6, Wisconsin	10
H. J. Phaff, Davis, California	1.1
Gustav Butschek, Wandsbeker Zoolstrasse 59, Germany	13
Brief News Items	13
Letters to the Editor	15

Many thanks to those who have contributed recently to help finance the Yeast News Letter. Because costs of mailing, mimeographing etc. increase continually, our treasurey is in rather poor shape. Receipts have not been sufficient to cover publication costs. It would be greatly appreciated if those readers which have contributed rarely or never to the News Letter would send \$0.50 or more to help finance future issues. We also would like to obtain a notice from those who are no longer interested in receiving the News Letter.

The next issue will be in November 1959

The Editors

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

Since our last report in the Yeast News Letter, the following new species (for which a description has been published) have been received by the C.B.S.

Candida olivarum Santa Maria

J. Santa Maria, Anales Inst. Nac. Invest. Agronom., No. 38, 301, 1958.

Candida polymorpha Ohara et Nonomura

Y. Ohara and H. Nonomura, J. Agr. Chem. Soc. Japan, 28, 717, 1954.

Candida vanriji Capriotti

A. Capriotti, Archiv. Mikrobiol., 30, 226, 1958.

<u>Debaryomyces</u> <u>konokotinae</u> Kudriavzev

Debaryomyces tamarri Ohara et Nonomura

Y. Ohara and H. Nonomura, J. Agr. Chem. Soc. Japan 28, 837, 1954.

Dioszegia hungarica Zsolt

J. Zsolt, Botanikai Kózlemenyek, 47, 63, 1957.

Saccharomyces aceti Santa Maria

J. Santa Maria, Nature, 182, 937, 1958.

Saccharomyces oleaceus Santa Maria

J. Santa Maria, Anales Inst. Nac. Invest. Agronom., No. 38, 301, 1958.

Saccharomyces oleaginosus Santa Maria

J. Santa Maria, Anales Inst. Nac. Invest. Agronom. No. 38, 301, 1958.

Saccharomyces oxidans Santa Maria

J. Santa Maria, Nature, 182, 937, 1958.

Saccharomyces smittii Capriotti

A. Capriotti, Antonie van Leeuwenhoek, 24, 215, 1958.

Sporobolomyces coralliformis Tubaki

Tubaki, Bot. Mag. Tokyo, 71, 133, 1958.

Torulopsis ingeniosa di Menna

M.E. di Menna, J. gen. Microbiol., 19, 581, 1958.

Torulopsis pseudaeria Zsolt

J. Zsolt, Antonie van Leeuwenhoek, 24, 210, 1958.

Torulopsis wickerhamii Capriotti

A. Capriotti, Archiv. Mikrobiol., 30, 383, 1958.

Zygosaccharomyces mrakii Capriotti

A. Capriotti, Archiv, Mikrobiol., 30, 387, 1958.

The following paper has been published:

"The relationship between <u>Saccharomyces tellustris</u> and <u>Candida bovina</u>" Antonie van Leeuwenhoek, <u>24</u>, 137, 1958. N.J.W. Kreger-van Rij

II. Communication from Prof. O. Verona (Istituto di Microbiologia Agraria e Tecnica, Facolta Agraria dell'Universita, Via S. Michele degli Scalzi 4 bis, Pisa, Italia) and Dr. A. Montemartini (Istituto Botanico dell' Universita, Casella Postale 165, Pavia, Italia).

The authors have reviewed and summarized the taxa described or modified since the appearance of the monograph by Lodder and Kreger-van Rij. The survey (up to 1958) includes the following genera, species and varieties:

1) genus Citeromyces (Santa Maria, 1957) with 1 species;

- 2) " Debaryolipomyces (Ramirez, 1957) with 2 species:
- 3) " Debaryomyces with 7 species, 1 variety and 2 n.comb.
- 4) " Endoblastomyces (Odinzova 1947) with 1 species;

5) " Endomyces with 1 species;

- 6) " Endomycopsis with 1 species and 1 variety;
- 7) " Fabospora (Kudriavzev, 1954) with 2 species;
- 8) " Hanseniaspora with 1 species, 2 n.comb. and one elimination of one species for synoymy;
- 9) " Hansenula with 9 species, 1 variety and 2 n.comb.
- 10) " Issatchentia (Kudriavzev, 1954) with 1 species;
- 11) " Kluyveromyces (van der Walt, 1956) with 2 species;
- 12) " Lipomyces with elimination of one species for synonymy;
- 13) " Metschnikowiella with 2 new combinations;
- 14) " Nadsoniomyces (Kudriavzev, 1932) with 1 species;
- 15) " Octosporomyces (Kudriavzev, 1954) with 2 new combinations;
- 16) " Pachysolen (Boidin et Adzet, 1957) with 2 species;
- Pichia (and discussion on genus <u>Petasospora</u> Boidin et Abadie, 1954 and <u>Dekkeromyces</u> Wickerham et Burton, 1956) with 14 species, 2 varieties;
- 18) " Saccharomyces with 21 species, 13 varieties;
- 19) family <u>Saccharomycodaceae</u> (Kudriavzev, 1954) with genus <u>Saenkia</u> (Kudriavzev, 1954);
- 20) genus Schizosaccharomyces with 1 species;
- 21) " Schwanniomyces with 2 species;
- 22) " Sporobolomyces with 3 species;
- 23) " Torulaspora with 2 species;
- 24) " Zygofabospora (Kudriavzev, 1954) with 1 species and 1 new combination
- 25) " Zygopichia (Kudriavzev, 1954) with 2 n. comb.
- 26) " Zygosaccharomyces with 6 species and 2 varieties;
- 27) " Zygowillia with 3 n. comb.

Among the asporegenous yeasts are listed:

- 1) genus Brettanomyces with 4 species and elimination of 2 species for synonymy;
- 2) " Candida with 19 species, 10 varieties, elimination of 2 species for synonymy and 3 with an ascosporic stage of the genera Endomyces and Endomycopsis;
- 3) " Cryptococcus with 3 species and 1 variety;
- 4) " Cystidiella (Malan, 1941) with 1 species;
- 5) " Kloeckera with 1 species and 1 variety;
- 6) " Pityrosporum with 1 species;
- 7) " Rhodotorula with 8 species;
- 8) " Torulopsis with 11 species, 2 varieties and several ascosporic stages;
- 9) " Trichosporon with 8 species and 1 variety;

A number of reprints are available; about 110 pages, in the italian language. Price \$1.-. Please write to the Botanical Institute, the University, P. O. Box 165, PAVIA, Italy.

III. Dr. Wm. Bridge Cooke, U. S. Public Health Service, Cincinnati, Ohio, pointed out the following article to the Editor, as he thought it to be of interest to readers of the Yeast News Letter. An abstract follows:

J. Zsolt, Institute for Plant Physiology, University, Szeged, Hungary. The Evolution of Domesticated yeasts, and some related problems. Acta Botanica: Academiae Scientiarum Hungaricae. 5(1-2): 233-257. 1959. With a bibliography of 79 titles.

The writer recognizes as yeasts the genera listed by Lodder and Kreger-van Rij (1952) and by Kudriavzev (1954, cf. Yeast News Letter Vol V, no. 1).

They are a heterogeneous group, which owes its existence to the common methods of study. They include the unicellular fungi and the fungi intermediate between these and the filamentous forms.

In taxonomical and phylogenetical investigations of microorganisms serious problems are inherent in the fact that observations can only be made in cultures, while the properties to be studied are gravely affected by the culture conditions; sometimes to the extent where it depends entirely on them whether or not a certain character will be perceived at all. It cannot be decided on a basis of principle what diagnostic feature is to be studied, or what experiment is to be designed in order to ensure perception of this or that property.

Every yeast investigator studies the fermentation and assimilation of various sugars. It seems desirable to increase the number of compounds to be studied for practical use in the industries. In the present situation of our branch of science this appears to be the route promising the detection of a number of taxonomically and phylogenetically really important diagnostic features.

All the current systems are more or less artificial, yet we cannot do without them if we are to keep our data in order.

Into the natural system the yeasts must be incorporated on a phylogenetical basis. It is very difficult to investigate the phylogenesis of the microorganisms. For the lack of fossil remains we have to rely on the recent forms when inferring the evolutionary process by means of phylogenetic lines. The microorganisms offer increased possibilities for experimental research into evolution, since the generations are short-lived and the organisms very ready to respond to external influences.

The timely tasks of yeast taxonomy and phylogeny are the following viz.:

(i) Detection of new yeasts.

(ii) Formation of homogeneous groups of known forms.

(iii) Tracing the origin of the individual properties.

(iv) Construction of the family tree within small groups.

The environment which gives rise to and directs the evolution of domesticated yeasts is determined by the various fermentation method which man employs. The earliest forms to develop were the brewers' and the wine yeasts. The distillery yeasts were later ones. The latest yeasts are the fodder and fat yeasts, directed in their development by man's present-day knowledge of nicrobiology.

Another paper is: No inhibition of pigment production by diphenylamine in <u>Candida pulcherrima</u> (lindner) Windisch. J. Zsolt and L. Ferenczy. Acta Biologica. Nova Series IV (1-2) 65-66, 1958, Szeged (Hungaria).

IV. Institute for Fermentation. Juso-Nishino-cho, Higashiyodogawa-ku, Osaka, Japan. Communicated by Dr. T. Hasegawa.

For several years, the author has concerned himself with a taxonomic study of Rhodotorula yeasts, attaching importance to correcting the type cultures maintained in Japanese collections.

Forty-six cultures of <u>Rhodotorula</u> which were lineal descendants from 12 old species published by Dr. Saito in 1922 and Dr. Okunuki in 1931 were examined together with authentic cultures of <u>Cryptococcus ruber</u>, <u>Rhodotorula glutinis</u>, <u>Torula mucilaginosa etc.</u> The cultures included several groups, each of which consisted of cultures from a strain of the above old species. Remarkable differences in cell morphology were often found between cultures of the same group. This dimorphism afforded a sound explanation for the discrepancies between the diagnosis by Drs. Lodder and Kreger-van Rij and those by the original authors. (The Meeting of the Society of Fermentation Technology, Osaka in 1955; J. Ferm. Techn. Vol. 34, No. 2, 1956., Vol. 36, No. 5, 1958). A similar result was reported independently by Drs. Skinner and Huxley in an article concerned with many wild species of <u>Rhodotorula</u> isolated by them. (Mycologia, Vol. 48, No. 3, 1956). From the above results, the author concluded that <u>Rhodotorula</u> aurantiaca is the same species as <u>Rhodotorula</u> glutinis, and <u>Rhodotorula</u> mucilaginosa is similar to <u>Rhodotorula</u> rubra.

Thereafter, a new species assimilating both potassium nitrate and lactose was found, and it was named <u>Rhodotorula lactosa</u> nov. sp. (J. Gen. Appl. Microbiology, Vol. 5, No. 1, 1959.) This species differs from <u>Rhodotorula macerans</u> by Dr. Sonne Frederiksen in four points; the iodine reaction produced by a starch-like compound, cell morphology, ethanol assimilation and their vitamin requirements. (Essentially, <u>Rh. macerans</u> required biotin and <u>Rh. lactosa</u> requires p-aminobenzoic acid.)

Since then, the author has been interested in the phylogenetic relationship between Rhodotorula species. Recently, there were found two phylogenetic lines related to vitamin requirements, that is, Rhodotorula glutinis (autotrophic) - Rh. glutinis var. dairenensis - Rh. rubra strain  $\propto$  (thiamine dependent line) and Rh. lactosa - Rh. marina (Rh. tokyoensis) - Rh. texensis - Rh. minuta - Rh. pallida (PABA dependent line). (J. Ferm. Techn. Vol. 37, No. 5, 1959).

V. <u>University of Kentucky, Lexington, Kentucky, Department of Zoology.</u> <u>Communicated by Dr. John M. Carpenter.</u>

The research started several years ago at the University of Kentucky on the role of yeasts in the feeding habits of <u>Drosophila</u> continues, with the first phase of the work having been completed. This phase, largely accomplished by a graduate student, Mr. James K. Komatsu, and dealing with the yeasts ingested by three <u>Drosophila</u> species — <u>affinis</u>, <u>putrida</u> and <u>robusta</u> during the various seasons of the year, has shown, on the basis of yeasts isolated from their crops, that these three species of <u>Drosophila</u> ingested essentially the same yeast species throughout the year. It was also discovered that yeast species common in Kentucky, and in Brazil (S.A.) were almost identical, but differed greatly

from California species. Publication of these results is contemplated. Mr. Komatsu will study at Cornell University next year with Dr. Adrian Srb.

VI. University of Miami, The Marine Laboratory, #1 Rickenbacker Causeway, Virginia Key, Miami 49, Florida. Communicated by Dr. Samuel P. Meyers.

Yeast Studies in Progress at the Marine Laboratory ....

Under a National Science Foundation research grant, beginning February, 1958, studies have been made on the occurrence and characteristics of the marine yeasts in Biscayne Bay, Florida, and adjacent subtropical marine localities. This work has been carried out under the direction of Dr. Samuel P. Meyers, Research Assistant Professor. Two graduate students, Mr. Jack Fell and Mr. Donald Ahearn, have conducted various aspects of this research, leading to their degree, Master of Science in Marine Biology.

Studies to date have shown the following summarized information:

- 1) Species of yeasts are abundant in sediments and ocean water, with different areas yielding dissimilar types of yeasts. The most abundant "pink yeasts" isolated have included Rhodotorula mucilaginosa and Rhodotorula glutinis. In all, over 300 isolates of yeasts have been collected, the majority of which fall into the family Cryptococcaceae. The genera identified to date include, Candida, Trichosporon, Rhodotorula, Cryptococcus, Pullularia, Hansenula, and Debaryomyces. The genera Candida and Rhodotorula especially are well represented. The most widely distributed species of Candida is C. tropicalis, closely followed by C. parapsilosis. To date, there is no indication of an indigenous marine yeast flora, although some species have been collected only in pelagic areas while others are common inhabitants of inshore or brackish localities. Studies are in progress to ascertain if marine strains of these species exist.
- 2) An incubation enrichment culture technique has been developed which permits the selective isolation of yeasts from sediments and various types of trapping materials. This procedure, incorporating antibiotics and a rich nutrient medium into the methodology, is in process of being described for subsequent publication.
- Dr. L. J. Wickerham has very kindly assisted in confirming many of the identifications, and has generously offered invaluable suggestions.
- VII. University of California, Donner Laboratory, Berkeley 4, California.

  Communicated by Dr. T. R. Manney.

Effects of Dehydration and Anoxia on Yeast Radiosensitivity to Densely Ionizing Particles

Thomas R. Manney, Tor Brustad, June Barr and C. A. Tobias Donner Laboratory, University of California, Berkeley, California

This paper was read at the Radiation Research Society Meeting, Pittsburgh, May 19, 1959.

To expand on results obtained by Alburt M. Rosenberg of the University of Pennsylvania, effects of dehydration with glycerol solutions and of anoxia

on the radiosensitivity of haploid yeast were studied over a range of linear energy transfer (LET) values in the range of 40 - 5000 Mev/g cm<sup>-2</sup>. Stripped nuclei of He, C, O, and Ne from the Berkeley heavy ion linear accelerator (HILAC) and x rays were used. Haploid Saccharomyces cerevisiae were irradiated on millipore filters in equilibrium with 6.9 M glycerol solution or, for controls, with M/15 KH2PO4. Throughout irradiation the chamber enclosing the samples was flushed with water-saturated air, 0, or N<sub>2</sub>. Viability was assayed by counting microcolonies after 24-hour incubation at room temperature; cells which formed colonies of more than ten cells were scored viable. Cells treated with glycerol were less sensitive than control samples by a factor or two or more for all LET values studied. Dose reduction factors (DRF), determined from exponential survival curves, were higher for the less densely ionizing alpha particles and x rays. The effect of anoxia paralleled the dehydration effect. Anoxia, however, gave smaller DRF throughout the LET range which were difficult to resolve for the more densely ionizing particles (DRF 1.2). Possible relationship between these two effects is still being studied. (Work supported by USAEC and ICA in association with the Norwegian Cancer Society and the U.S. National Academy of Sciences.)

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

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

Lindegren, C. C., Lindegren, G., Shult, E. E. and Desborough, S. Chromosome maps of Saccharomyces. Nature 183: 800 (1959).

Lindegren, Carl C., Lindegren, G. and Desborough, S. Gene controlled resistance vs sensitivity to caffeine and nicotine in Saccharomyces. J. Gen. Microb. Accepted for publication.

Yuasa, Akira and Lindegren, C. C. The integrity of the centriole in Saccharomyces. Antonie van Leeuwenhoek. Accepted for publication.

Lindegren, C. C. and Pittman, D. The "all-or-none" nature of X-ray induced backmutation at the melezitose locus in Saccharomyces. Genetica. Accepted for publication.

On March 20, 1959, Dr. Lindegren gave a talk entitled "The Centriole as the primary radiation target in the resting cell" at the New York Academy of Science meeting. This paper will be published in the Proceedings of this Society.

- IX. Western Reserve University, Cleveland 6, Ohio, Biological Laboratory. Communicated by Dr. Thomas D. Brock.
- 1. Change of address. Beginning in the Fall of 1959, my new address will be: Dept. of Microbiology, School of Medicine, Western Reserve University, Cleveland 6, Ohio.
- 2. As indicated in early news letters, the species <u>Hansenula wingel</u>, discovered by Wickerham, is especially useful for studying some of the physiological processes involved in mating. Work has continued on the conjugation process in this yeast. When cells of the two mating types are mixed and aerated in a simple nutrient fluid containing only glucose, potassium phosphate and magnesium

sulfate, rapid conjugation and cell fusion occur, resulting in up to 70% conjugants in five hours. Microscopic studies in slide culture indicate that fusion occurs because each cell of a mating pair elongates on the side where it touches its opposite member. After several hours the cross wall between the cells disappears and at this time further elongation of the conjugants ceases when in non-growth medium. For fusion to occur, both cells must be metabolically active. This has been shown by the pairing of ultraviolet inactivated cells of one type with normal cells of the opposite type. Under these conditions, fusion does bud. This seems to innot occur, but the untreated cells are stimulated to dicate that conjugation is due to induction of a budding enzyme in the one cell type by contact with its opposite type, since the isolated mating types do not bud in this non-growth medium. The nutrient materials for the synthesis of these enzymes do not come from the medium, which is devoid of nitrogen compounds, but from the free amino acid pool of the cells. Conjugation can be markedly reduced by the depletion of this pool, and can be completely inhibited by the use of specific amino acid antagonists. These results indicate that conjugation may result from reciprocal induction in each cell of an enzyme which digests a portion of its own cell wall. Conjugation can therefore be viewed as an extension of normal budding processes, induced by contact with cells of opposite mating type. Thus the mating process in yeast does not require the postulation of hormones which diffuse into the medium, although the possibility of direct cellto-cell transfer of such hormones cannot be ignored.

Publication: Biochemical basis of mating in yeast, by T. D. Brock. Science, April 10, 1959. Vol. 129, 960-961.

# K. Illinois Institute of Technology, Biology Department. Communicated by Marilyn Kvetkas and L. R. Hedrick.

The growth responses to amino acids of <u>Kloeckera antillarum</u>, <u>Kl. javanica</u>, and <u>Kl. jensenii</u> have been measured turbidometrically. Each of the eighteen amino acids of casein were tested individually and supplied as the only source of nitrogen in synthetic media. <u>Kl. antillarum</u> and <u>Kl. javanica</u> exhibited only quantitative differences in their responses to these amino acids when they were compared. <u>Kl. jensenii</u> was completely unlike the other two organisms and grew only in the presence of each one of six anino acids.

Therefore, the lack of growth in response to one or more of the remaining twelve amino acids may be used as a taxonomic aid in conjunction with the criteria of Lodder and van Rij in identifying this species.

# XI. Laboratory of the Kodama Brewing Co., Ltd., Iitagawa-machi, Akita Prefecture, Japan. Communicated by Dr. K. Kodama.

The following are some further translated abstracts of the article: "Studies on film yeasts isolated in Japan" - Jour. Ferment. Technol. 1958 (80 pp) cf. Yeast Newsletter vol. VII pg. 24.

"Maximum tolcrable concentration of various compounds for the growth of certain yeasts".

10 strains belonging to <u>Pichia and Hansenula</u> isolated from samples related to the brewing industry, were used.

The test compounds (related to the brewing industry) were glucose, lacticacid, acetic acid, ethyl alcohol, sodium chloride.

Lodder's medium and Koji extract (10° Balling) was used as basal medium. Tests were carried out according to Carbon assimilation test recommended by Wickerham.

Results are shown in Table 26 (below).

Table No. 25

Strain/ compound in %	A	В	C	D	E
	glucose	Lactic acid	Acetic acid	Alcohol	NaCl.
H. anomala (Sake)	55-60	2.8-3.0	0.3-0.4	8-10	10-14
H. anomala form. X	<b>55-60</b>	3.0-3.2	0.3-0.4	9-10	10-14
P. sake form. 🗷	60 <b>~</b> 65	1.7-1.9	0.3-0.4	7-8	18-19
P. farinosa (orig)	6 <b>0+65</b>	1.7-1.9	0.4-0.5	8-10	14-15
H. anomala (soya)	50-55	3.0-3.2	0.3-0.4	7-8	10-14
P. membranaefaciens (orig) P. membranaefaciens var.	44-48	1.8-2.0	1.0-1.2	6-7	12-13
nandshurica P. membranaefaciens var.	44-48	3.0-3.2	1.2-1.4	<b>5−7</b>	12-13
belgica	44-48	2.8-3.0	1.2-1.4	8-9	12-13
H. saturnus	30-34	2.0-2.2	0.2-0.3	4-6	10-12
H. staveolens	24-28 gr/100cc	1.7-1.9	0.2-0.3	4-6 Vol %	10-12 gr/100cc

#### Discussion

### 1. Glucose and Sodium chloride

P. Sake form. A and P. farinosa are osnophilic yeasts which are able to grow in 60%-65%, 15%-18% of each solution, respectively. This property is one of the reasonswhy these strains are occasionally found in Soya sauce or Miso brewing process carried out in the presence of high concentrations of salt.

### 2. Acetic acid

P. membranaefaciens group can grow in relatively high concentrations of acetic acid i.e. 1.2-1.4%, pH 2.7-2.9. This property is considered one of the important reasons why this species is an occasional contaminant in the vinegar brewing process in Japan. Moreover this property is remarkably specific and was never observed in any other species of <u>Pichia</u> and <u>Hansenula</u> so far as the strains are concerned isolated by us.

# 3. Ethyl alcohol

All strains possess a general tendency to be able to grow in relatively high concentrations of ethyl alcohol (7-10 vol. %).

4. Members of the <u>H. anomala</u> group can grow generally in high concentrations of all compounds. I consider this a reason why this species is widely distributed in nature.

I would be very happy if any one would kindly inform me of publications or literature related to the above mentioned study.

XII. Microbiology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa. Dr. H. Katznelson Director. Communicated by G. B. Landerkin.

The nutrilite requirements of 20 osmophilic yeasts of the genus Zygosaccharomyces were redetermined after more than 12 years' laboratory culture with semi-annual transfer on 30% glucose-honey-yeast agar. The results to be published in detail later indicate that these yeasts represent a relatively stable group in that aside from minor deviations their vitamin requirements are as reported previously by Lochhead and Landerkin, J. Bact. 33, p. 343, 1942.

XIII. University of Illinois, Department of Bacteriology, 127 Biology Building, Urbana, Illinois. Communicated by Dr. F. M. Clark.

The only work which I can report for the Yeast News Letter from the University of Illinois is work that I have been doing on Torulopsis melibiosum. This yeast grows very nicely in a synthetic medium containing ammonium salts, inorganic salts, vitamins and with inositol as the sole source of carbon. Our problem has been to determine the intermediate and end products of inositol decomposition in this medium. The principal end products containing carbon have been the cells of the yeast and carbon dioxide. A small amount of pyruvic acid and lactic acid also appear in the medium during growth of this organism. So far we have been able to account for only 56% of the carbon added as inositol to the original medium. No indication as to intermediates has been obtained.

It may be of interest to some of the readers of the News Letter to know that we have moved into a new Biology Building. The Department of Bacteriology has offices, laboratories and research space on the first three floors of this building.

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

A team of chemists and biologists in our laboratory have produced a polymer designated as phosphomannan Y-2448 from <u>Hansenula holstii</u>, the perfect form of <u>Candida silvicola</u> Shifrine and Phaff. The phosphomannan in two percent concentration in water, plus a small amount of borax and potassium chloride, makes a very viscous, clear solution. It is hoped that this new polysaccharide from yeast may find use in the pharmaceutical, food, beverage, and tobacco industries.

Hansenula holstii is a heterothallic haploid yeast associated primarily with coniferous trees and bark beetles. It rarely forms diploids. A diploid was used for the preparation of phosphomannan because the cells are larger and they are generally more stable than the haploid. H. holstii will be described in Mycologia.

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

Since the last publication of the YEAST NEWS LETTER the following articles have been published or accepted for publication.

1. J. D. Duerksen and Harlyn Halvorson. The specificity of induction of B-glucosidase in Saccharonyces cerevisiae.

- 2. H. K. Kihara, A. S. L. Hu, F. Hoh and H. O. Halvorson. The presence of a normally soluble enzyme in purified ribosome preparations.
- 3. A. M. McQuillan, H. O. Halvorson and S. Winderman. On the constitutive synthesis of B-glucosidase in yeast.
- 4. Hariya Halvorson. The control of enzyme biosynthesis in microorganisms.

Dr. A. Hu and Mr. Epstein have purified the constitutive B-glucosidase from the hybrid <u>S. fragilis X S. Dobzhanskii</u>. The constitutive enzyme is identified in its substrate specificity, pH optimum, energy of activation, molecular weight and physical properties to the enzyme previously isolated from inducible "Yeast Foam". We are currently attempting to prepare antisera against the purified enzyme.

Mrs. I. Winicov has recently joined our group from Dr. J. Lederberg's laboratory. She has prepared a series of mutants either from the above hybrid or from the B-glucosidase producing parent of the cross (S. dobzhanskii). These mutants produce B-glucosidase constitutively at decreasing levels of efficiency. Mr. McQuillen and Miss S. Winderman have been examining these semi-constitutive strains. In all cases the addition of inducer raises the differential rate of enzyme synthesis to that of an inducible cell. The inducer response in the semi-constitutive strains varies in several important aspects from the inducible Yeas: Foam. We are currently studying the role of the inducer in these strains.

Mr. H. Kihara has been purifying ribosomes from constitutive, inducible and negative strains for B-glucosidase. All strains contain a firmly bound B-glucosidase which can be released by borate buffer or urea. We are currently examining this fraction for possible precursor relationships to enzyme synthesis.

Dr. Joel Mandelstam from Mill Hills, England joined our lab group last January. Dr. Mandelstam has been further examining the turnover of protein and nucleic acid in non-growing bacteria and yeast.

One of the problems we have recently faced is the preparation of ribosomes and enzymes in large quantities from yeast. This has involved the effective breakage of 5-10 pounds of yeast. In collaboration with Dr. J. C. Garver, Biochemistry Department, University of Wisconsin, Mr. R. L. Epstein has developed a new method employing "Superbrite" glass beads and an Eppenbach laboratory colloid mill. Under appropriate conditions over 99 per cent breakage has been obtained in 20 min. The system is equally effective for bacteria, Aspergillus and Myccobacterium. A brief note describing the condition and method has recently been submitted to the Journal of Bacteriology, "A Method for rupturing large quantities of microorganisms."

XVI. Department of Food Technology, University of California, Davis, California. Communicated by H. J. Phaff.

Dr. Harry E. Snyder has completed his Ph.D. dissertation and has accepted the position of Assistant Professor in the Department of Dairy and Food Industries at the University of Iowa, Ames, Iowa. A brief abstract of his thesis follows.

# STUDIES ON A BETA-FRUCTOSIDASE (INULINASE) PRODUCED BY SACCHAROMYCES FRAGILIS

It has been shown that the yeast <u>Saccharomyces fragilis</u> produces an enzyme which catalyzes the hydrolysis of the plant polysaccharide inulin. Inulin is a linear polymer composed of about 30 fructose units, joined by 2,1 beta-linkages, and a terminal glucose molecule, which is also linked by a 2,1 bond. The beta-fructosidase produced by <u>S. fragilis</u> also catalyzes the hydrolysis of sucrose, raffinose and of bacterial levans.

On the basis of substrate specificity the inulin-hydrolyzing enzyme of S. fragilis is similar to invertase of baker's yeast. However, the rate of hydrolysis of sucrose divided by the rate of hydrolysis of inulin was shown to be about 25 for the beta-fructosidase of S. fragilis but about 14,000 for invertase of baker's yeast. The higher relative rate of inulin hydrolysis by the enzyme of S. fragilis was the basis for naming this enzyme inulinase.

High yields of intra- and extracellular inulinase were produced by <u>S</u>.

<u>fragilis</u> during aerobic growth with inulin or raffinose as the carbon source and with amonium phosphate as the nitrogen source. Relatively low yields were obtained with glucose, fractose, or sucrose as the carbon source.

The inulinase content of the cells was generally greater than that found in the culture medium when expressed as activity per unit volume of culture medium. The extra- and intracellular inulinases were concentrated and purified to the same extent. Comparison of the two preparations with respect to substrate specificity, rate of inactivation by heat, pH optima with sucrose and with inulin as substrates, and elution patterns from a column of diethylaminoethyl cellulose indicated that the two enzyme preparations are identical.

Inulinase was found to attack inulin by a sequential hydrolysis of fructose molecules from the end of the chain opposite to that carrying the terminal glucose. The rate of appearance of free glucose was compared to that of total hexoses liberated during the enzymatic hydrolysis of inulin. These results showed that a single-chain mechanism was operative. A single-chain mechanism refers to the complete hydrolysis of one polymer molecule by an enzyme molecule as a result of a single enzyme-substrate encounter.

It was found that the rate of inulinase excretion paralleled closely the rate of cell division throughout the growth of <u>S. fragilis</u>. However, when the rate of cell division and enzyme excretion declined at the end of the logarithmic growth phase, intracellular inulinase accumulated in the presence of residual substrate. This indicated that enzyme continued to be synthesized but its excretion was inhibited. The excretion of inulinase was shown to be specific and could not have resulted from a breakdown of cells with concommittant release of protein. The conclusion was drawn that the mechanism of excretion of inulinase is linked with the budding process.

### Publications:

On the mechanism of action of yeast-endopolygalacturonase on oligo-galacturonides and ther reduced and oxidized derivatives. By D.S. Patel and H. J. Phafi. Jour. Biol. Chem. 234, 237 (1959).

Studies on the life cycle and nuclear behavior of a species of the yeast genus Schwannionyces. By J.D. Ferreira and H.J. Phaff. Jour. Bacteriology (in press).

XVII. <u>Dr. Gustav Butschek, Pirector of the Norddeutschen Hefeindustrie AG, Hamburg-Wandsbek, Wandsbeker Zoolstrasse 59, Germany, has communicated the following information.</u>

Standardization of dried yeast (cf. Yeast News Letter VII, pg 36, 1958).

The committee of the Int. Union of Pure and Appl. Chemistry for the establishment of standards for dried yeast and for the standardization of analytical procedures has met for the second time in October 1958 under chairman Prof. H. Lundin (Stockholm). It has worked out the following definition and characterization for dried yeast:

"By dried yeast is meant the dried whole organism of yeasts of the family Saccharomycetaceae, sub-family Saccharomycetoideae, and of the family Cryptococca-ceae, sub-family Cryptococcoideae (classification Lodder and Kreger-van Rij), obtained either as a by-product of fermentation processes or by special cultivation and conforming to such standards as may be laid down.

It is desirable that the origin of the yeast should be specified by the manufacturer.

It should not be permitted to sell under the label "Dried yeast" yeast products which have been extracted or to which fillers have been added.

Enrichment by addition of vitamins and/or amino-acids should be permitted, provided they are declared. "

Standards for the composition of dried yeast, in particular with respect to the minimum vitamin contant, were also discussed at this session. However, compulsary minimum values will be worked out during the next session to be held in August of this year in Munich. At that time the results of an analysis of a yeast sample by ten laboratories of various countries will be evaluated and a choice will be made of the most suitable methods for the determination of various constituents of dried yeast.

XVIII. Brief News Items and Recent Publications.

- 1. Dr. P. K. C. Austwick (Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England) writes: I am coming to the IXth International Botanical Congress at Montreal in August and hope to follow this with a short tour of research establishments in Canada and the Eastern United States. Regrettably I shall not be able to visit the West but hope to meet many of my North American colleagues during my tour.
- 2. Dr. Emil M. Mrak will become Chancellor of the Davis campus of the University of California on July 1, 1959. We congratulate Dr. Mrak with this high honor and will miss him as an active "yeastologist". The departments of Food Technology and Dairy Science will be combined into one department under the name "Department of Food Science and Technology" and Dr. George F. Stewart will be the new Chairman. Dr. Stewart was formerly head of the Department of Poultry Husbandry on the Davis campus.
- 3. Dr. Edward D. DeLamater (University of Pennsylvania, Philadelphia 4) writes: Dr. Giovanna R. Mazzanti, who has worked with me for the last two years, has accepted a position in the laboratory of the Skin and Cancer Hospital of Philadelphia, in which I also do some consulting. I am to present a lecture

entitled, "The Use of Drugs in the Analysis of Cell Structure" at the Wellcome Research Laboratories on June 4, and will present a paper at the International Botanical Congress in Montreal entitled, "Observations on the Isolated Nuclei and Chromosomes of B. megaterium by Cytochemical and Electron Microscopic Techniques". One of my students, Mr. Patrick Echlin, will likewise present a paper at the same Congress entitled, "The Cytochemistry of the Nucleus of B. megaterium". After this I will present a paper entitled, "Observations on the Organization of the Isolated Bacterial Chromosome", at the Genetics Society meeting at Pennsylvania State University.

4. Dr. C. Ramirez (Instituto Jaime Ferran" de Microbiologia Joaquin Costa, Madrid, Spain) writes: The following two papers from our laboratory will appear soon.

"Nouvelle methode simple et rapide pour déterminer le degré de fermentation du raffinose par les levures" will come out in Antonie van Leeuwenhoek.

"Nouvelle diagnose d'une levure isoleé d'un Amanita livido palescens", which will appear in Revue de Mycologie, Institut de Cryptogamie, Muséum National d'Histoire Naturelle de Paris.

The last paper deals with a new species originally described and named <u>Schizosaccharomyces</u> <u>zambettakesi</u>, but now reclassified under the name <u>Geotrichum zambettakesi</u>.

- 5. The annual "Round Table Discussion Group on Yeasts" met this year in May during the National S.A.B. Meetings in St. Louis. About 75 people attended the yeast session, which was held at the Anheuser Busch Brewery. We wish to thank our hosts for supplying a meeting room and for the excellent food and refreshments. Problems dealing with taxonomy, ecology, genetics, cytology and physiology were discussed for several hours under the chairmanship of H. J. Phaff.
- 6. Recent publications received from readers of the News Letter (Some of the publications are of older date, but since they were published in relatively little known journals they are included here).

The Coexistence of Haploidy and Diploidy in Yeasts. Experientia Vd. XV/3, p. 99 1959. J. A. Barnett, Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge, and Department of Scientific and Industrial Research, Cambridge.

Complementary Genes Controlling Homothallism in <u>Saccharomyces</u>. Genetics, Vol. 43, No. 5 September 1958. Toshiaki Takahashi, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan.

A Method for the Rapid (Serological) Identification of the Genus Candida. Mycopathologia et Mycologia Applicata. Takeshi Tsuchiya, Yoshimura Fukazawa and Sukeyuki Kawakita, Department of Bacteriology, School of Medicine, Juntendo University, Tokyo. (References to previous papers on this subject are given)

Studies of the Fine Structure of Microorganisms IV. Observations on Budding Saccharomyces cerevisiae by Light and Electron Microscopy. Journal of Bacteriology Vol. 77, No. 3 pp. 344-354, March, 1959. Tadayo Hashimoto, S. F. Conti, and H.B. Naylor. Laboratory of Bacteriology, Cornell University, Ithacs, New York.

A New Variety of <u>Pullularia fermentans</u> Wynne et Gott. The Botanical Magazine, Tokyo. Vol. 72, No. 849, March 1959. Minoru Yoneyama, Biological Laboratory, Minami-Bunko, Hiroshima University, Hiroshima, Japan.

Taxonomic Considerations on some <u>Candida Yeasts</u>. Revista Latinoamericana De Microbiologia, Vol.1Num. 3, 30 De <u>Sept. 1958 pg. 233.A. Sanchez-Marroquín</u>, Escuela Nacional de Ciencias Químicas, Ciudad Universitaria, Mexico 20, D.F.

Yeasts from the Leaves of Pasture Plants. New Zealand Journal of Agricultural Research, Vol. 2, No. 2 April 1959. M. E. Di Menna, Soil Bureau, Department of Scientific and Industrial Research Wellington.

The Life Cycle of Kluyveromyces Polysporus. Comptes Rendus Des Travaux Du Laboratoire Carlsberg, Vol. 31 No. 9. C. Roberts and J. P. Van Der Walt.

The Wine Yeasts of the Cape Part I. - A Taxonomical Survey of the Yeasts Causing Turbidity in South African Table Wines. Antonie van Leeuwenhoek 24,239 1958. J. P. Van Der Walt and Amelia E. Van Kerken, National Chemical Research Laboratory, South African Council for Scientific and Industrial Research, Pretoria, South Africa.

Studies on the genus Pityrosporum in Japan. Nagaoa No. 4, September 1954. Kiyosi Kominami & Masami Soneda, Nagao Institute, Kitashinagawa, Tokyo.

Yeasts and Moulds in the Trunk-Exudations. Bulletin of the National Science Museum, No. 33, July, 1953. Yoshio Kobeyashi, Mational Science Museum, Tokyo.

Marine Yeasts Isolated from Little-neck Clam. Bulletin of the National Science Museum, No. 33, July 1953. Y. Kobayashi, K. Tsubaki and M. Soneda, National Science Museum, Tokyo.

The Yeast Nadsonia in Japan. The Journal of Japanese Botany, Vol. 32 No. 11 November 1957. Masami Soneda, Nagao Institute, Kitashinagawa, Tokyo.

7. Professor A. Chaves Batista (Universidade Do Recife, Instituto De Micologia, Recife, Pernambuco, Brasil) writes: It is a pleasure for me to inform the readers of the Yeast News Letter that I am doing in the Institute of Mycology, University of Recife, Pernambuco, Braxil, intensive work with yeasts of medical interest, involving the collaboration of Dr. Jarbas Sizenando Silveira and Reginaldo Pessoa Coélho.

We are working on the incidence and pathogenicity of yeast like fungi into the human digestive system skin and vagina. I have now studied systematically more than two thousand strains of these fungi.

The most recent findings are the incidence of <u>Hanseniaspora valbyensis</u> Klocker on the human appendix and also skin, the study of vaginal cryptococcosis caused by <u>Cryptococcus albidus</u> (Saito) Skinner, <u>C. diffluens</u> (Zach.) Lodder & van Rij and <u>C. laurentii</u> (Kuff.) Skinner, as well the occurrence of a new species <u>Schwanniomyces hominis</u> Bat. Vieira & Coelho as the agent of an epidermal lesion.

IXX. Letters to the Editor

Dear Sir:

I enjoyed very much our meeting at Anheuser-Busch, and I believe that your conduct of the discussion was marvelously successful. I know that every city in which the SAB meets supports a brewery which would be more than happy, as the people at Anheuser-Busch were, to act as hosts for the Round Table. I would like to suggest that Elsie Singruen, or someone else prominently connected with the brewing industry, would be requested to make contacts with such breweries and apprise them of our Round Table and the problems involved.

Carl C. Lindegren, Director Biological Research Laboratory Carbondale, Illinois