Y E A S T

A News Letter for Persons Interested in Yeast

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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 1962. A contribution of $0.50 from those who have not contributed for some time would be appreciated to finance future editions of the News Letter. Many thanks to those who have contributed recently.

The Editors

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

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

Debaryomyces cantarellii Capriotti
(A. Capriotti, Arch. Mikrobiol., 39: 123, 1961).

Debaryomyces fukuyamaensis Naganishi

Endomycopsis scolyti Phaff et Yoneyama
(H. J. Phaff & M. Yoneyama, Antonie van Leeuwenhoek, 27: 196, 1961).

Hansenula angusta Teunisson, Hall et Wickerham
(D. J. Teunisson, H. H. Hall & L. J. Wickerham, Mycologia, 52: 184, 1960).

Hansenula holstii Wickerham
(L. J. Wickerham, Mycologia, 52: 171, 1960).

Metschnikowiella krissii v. Uden et Castelo-Branco
(N. van Uden & R. Castelo-Branco, J. Gen. Microbiol., 26: 141, 1961).

Saccharomyces cerevisiae Hansen var. pelliculosa Goto

Saccharomyces osmophilus Barre et Galzy
(P. Barre & P. Galzy, Ann. Tech., 4: 345, 1960).

Torulopsis cantarellii v.d. Walt et v. Kerken
(J. P. van der Walt & A. E. van Kerken, Antonie van Leeuwenhoek, 27: 206, 1961).

Torulopsis capsuligenus v.d. Walt et v. Kerken
(J. P. van der Walt & A. E. van Kerken, Antonie van Leeuwenhoek, 27: 206, 1961).

Torulopsis castellii Capriotti
(A. Capriotti, J. Gen. Microbiol., 26: 41, 1961).

Torulopsis vanzylii v.d. Walt et v. Kerken
(J. P. van der Walt & A. E. van Kerken, Antonie van Leeuwenhoek, 27: 206, 1961).

Wickerhamia fluorescens Soneda
(M. Soneda, Nagaoa, 6: 1, 1959;
M. Soneda, Nagaoa, 7, 9, 1960).

Metschnikowiella zobellii v. Uden et Castelo-Branco
(N. van Uden & R. Castelo-Branco, J. gen. Microbiol., 26, 141, 1961).

II. Arthur Guinness Son & Company, St. James's Gate, Dublin, Ireland. Communicated by Dr. R. B. Gilliland.

The genus Brettanomyces, which consists of small slow-growing yeasts, is generally associated with fermented beverages. While these yeasts may produce a desirable and characteristic flavor in some very strong beers, they can be a cause of spoilage in beers of normal gravities. Strains isolated from naturally conditioned beers with off-flavors were classified into three species, Brett. bruxellensis, Brett. schanderlii, and an unidentified species. As the unidentified species fermented some sugars very slowly, methods for the detection of these slow fermentations were compared and their taxonomic significance was considered. The characteristics of this yeast warranted its designation as a new species, for which the name Brett. dublinensis was proposed. It fermented glucose, fructose, mannose, galactose, sucrose, lactose, and cellobiose at a normal rate for Brettanomyces and it fermented maltose, trehalose, and raffinose very slowly. These slow fermentations were not due to mutation, nor could the yeast adapt to rapid fermentation of these sugars on repeated subculture in media containing them.

R. B. Gilliland, Brettanomyces I. Occurrence, characteristics, and effects on beer flavor. Journ. Inst. Brew. 67, 257, 1961.

R. B. Gilliland, Brettanomyces II. The taxonomic significance of slow fermentations and description of a new species (In press).

III. South African Council for Scientific and Industrial Research, P. O. Box 395, Pretoria, South Africa. Communicated by Dr. J. P. van der Walt.

I would like to announce the forthcoming publications in Antonie van Leeuwenhoek of the following new species:

Brettanomyces custersianus nov. spec.
Cells in malt extract after 5 days at 25°C: Short oval, frequently ogive, long-oval to very much elongated, single, pairs or short chains, (1.2 - 3.8) x (2.5 - 15 - 27)µ.
Pellicle formation.
Pseudomycelium abundantly produced.
Only glucose and trehalose fermented.
Only glucose trehalose, saccharose (weakly) ethanol, glycerol, potassium D-gluconate, DL-lactic acid and succinic acid are assimilated.
No utilization of either nitrate or nitrite.
Acid production from glucose: Positive.
Like other members of the genus, the new species also displays a 'Negative Pasteur Effect'.
Isolated from brewing equipment in Bantu beer breweries.

Schwanniomyces persoonnii nov. spec.
Cells in malt extract after 3 days at 25°C: Short-oval to long-oval, in pairs, short chains of clusters (3 - 8) x (4 - 10)µ.
No characteristic pseudomycelium.
1 - 2 worty ascospores usually with a thin equatorial ledge are formed per ascus.
Glucose, saccharose and raffinose (Y3) fermented - fermentation of trehalose and melizitose variable.

Glucose, saccharose, maltose, trehalose, melizitose, raffinose, ethanol, adonitol (variable), D-mannitol, D-sorbitol, α-methyl-D-glucoside, potassium D-gluconate, succinic acid (weak) and citric acid are assimilated.
Nitrate and nitrite not utilized.
This species appears to be closely related to Schw. occidentalis but differs from the latter in so far as that galactose and xylose are not utilized.
The species has been named for C. H. Persoon (1762-1836) on the advent of the bicentenary of his birth. The species was isolated from soils of Transvaal.

Candida ingens
Cells in malt extract after 3 days at 20°C: Large, round, oval to cylindrical, single in pairs clusters or chains, (5 - 9) x (7 - 13 - 28)μ.
Pellicle formation. Primitive pseudomycelium.
Fermentation absent.
Only glucose, galactose (latent), L-sorbose (latent), ethanol, glycerol, DL-lactic acid and succinic acid are assimilated. No utilization of either nitrate of nitrite.
The species was isolated from malted Sorghum and from winery and brewery equipment.

IV. Dr. J. Zsolt, Dept. for Plant Physiology, University of Szeged, Hungary, has sent us the following publication: "A new system proposed for yeasts", by E. K. Novak and J. Zsolt, Acta Botanica Acad. Scient. Hungaricae 7, 93-145, 1961. Dr. Wm. Bridge Cooke, U. S. Public Health Service, Cincinnati, Ohio, has prepared the following key based on the system proposed by Novak and Zsolt. The genera in parentheses are considered synonymous to those given above.

Key to Families, Subfamilies and Genera According to Novak and Zsolt

Order: Endomycetales

1. Ascospores produced 2
1. Ascospores not produced 5
   2. Exozygotic ascus formation . . . . . Lipomycetaceae Novak & Zsolt

Nadsonia Sydow
Pachysolen Boidin & Adzet
Lipomyces Lodder & Kreger-van Rij
Debaryolipomyces Ramirez

   2. Holozygotic ascus formation 3
3. Cell formation by splitting . . . . . Schizosaccharomycetaceae Klöcker

Endomyces Reess.
Schizosaccharomyces Lindner

3. Cell formation by budding 4
4. Ascospores globose or slightly ovoid . . . . Saccharomycetaceae emend.

a. With true mycelium . . . . . Prosaccharomycetoideae N. & Z.
Prosaccharomyces N. & Z.
(Endomycopsis Dekker pro p.)

b. With 1-many ascospores . . . Multisporoideae N. & Z.
Endoblastomyces Odinzova
Nadsoniomyces Kudriavzev

c. Ascospores smooth . . . . . Laevigatosporoideae N. & Z.

(1) No fermentation, no assimilation of nitrates . . .
Azymomyces N. & Z.

(2) Fermentation, no assimilation of nitrates . . . .
Saccharomyces Meyen. emend.
(Saccharomyces emend.
Reess. p.p.)

(3) Fermentation, assimilation of nitrates . . . . .
Pseudohansenula N. & Z.
(Hansenula H. & R. Sydow p.p.)

(4) Other genera in this subfamily . . . Torulaspora Lindner
Saccharomycopsis
Schiönning
Saccharomycodes
Hansen

d. Spores worty . . . . . Verrucosporoideae N. & Z.

(1) Cells globose or ovoid, no fermentation . . . . .
Debaryomyces ss.
L. & K-R. emend.
(Debaryomyces ss.
L. & K-R. p.p.)

(2) Cells globose or ovoid, fermentation . . . . .
Zymodebaryomyces
N. & Z.
(Debaryomyces
sensu L. & K-R.
p.p.)

(3) Cells lemon shaped . . . . . Vanderwaltia N. & D.

4. Ascospores hemispherical, hat-shaped or Saturn-shaped . . Hansenulaceae
N. & Z.

a. With true mycelium . . . . . Prohansenuloideae N. & Z.
Endomycopsis Dekker emend.
(Endomycopsis Dekker p.p.)

b. No nitrate assimilation . . . . . Pichioideae N. & Z.

(1) No fermentation . . . . . Pichia Hansen emend.
(Pichia Hansen p.p.)

(2) Fermentation . . . . . Zymopichia N. & Z.
(Pichia Hansen p.p.)

(3) Also placed in this family. Hanseniaspora Zikes
Schwanniomyces Klöcker

c. Assimilation of nitrates . . . . Hansenuloideae N. & Z.

(1) No fermentation . . . . . Azymohansenula N. & Z.
(Hansenula H. & P. Sydow p.p.)

(2) Fermentation . . . . . Hansenula H. & P. Sydow emend.
(Hansenula H. & P. Sydow p.p.)

4. Spores reniform or oblong-ovoid, smooth . . . Fabosporaceae N. & Z.

Guilliermondella Nadson & Kudr.
(Endomycopsis Dekker p.p.)
Kluyveromyces van der Walt
Dekkeromyces Wick. & Burt.
(Zygofabospora Kudr.)
(Fabospora Kudriavzev.)

4. Ascospores spindle-shaped . . . . . Nematosporaceae N. & Z.

Nematospora Peglion
Metschnikowiella Genkel
Coccidiascus Chatton

5. Ballistospores produced . . . . . Sporobolomycetaceae Derx

(1) True mycelium occurs . . . . . Prosporobolomyces N. & Z.
(Sporobolomyces K. & v. N. p.p.)

(2) No true mycelium . . . . . Sporobolomyces K. & v. N. emend.
(Sporobolomyces K. & v.N. p.p.)

(3) Also assigned to this family . . . . . Bullera Derx

5. Budding cells or budding cells and arthrospores or only arthrospores are present. True mycelium and pseudomycelium may form. Cells generally hyaline, dark color or carotenoid pigments may be present . . . . .
Cryptococcaceae L. &. K.-v.R. emend.

a. Arthrospores and true mycelium always present, pseudomycelium and budding cells may occur . . . . . Trichosporoideae L. & K-v.R. emend.
(Trichosporoideae L. & K.-v.R. p.p.)

(1) Arthrospores only . . . . . Geotrichum Lk.
(Oospora Wallr.)
(Oidium Lk.)

(2) No fermentation . . . . . Trichosporon Behrend. emend.
(Trichosporon behrend p.p.)

(3) Fermentation . . . . . Fermentotrichon N. & Z.
(Trichosporon Behrend p.p.)

b. No arthrospores. True mycelium occurs, pseudomycelium may occur . .
Procahdidoideae N. & Z.

(1) No fermentation . . . . . Azymoprocandida N. & Z.
(Candida Berkhout p.p.)

(2) Fermentation . . . . . Procandida N. & Z.
(Candida Berkhout p.p.)

c. No arthrospores, no true mycelium, pseudomycelium occurs . . . .
Candidoideae N. & Z.

(1) No fermentation . . . . . Azymocandida N. & Z.
(Candida Berkhout p.p.)

(2) Fermentation . . . . . Candida Berkhout emend.
(Candida Berkhout p.p.)

(3) Also placed here . . . . . Brettanomyces K. & v.L.

d. No true mycelium and no pseudomycelium. Fermentation . . . . .
Torulopsoideae N. & Z.

(1) Fermentation . . . . . Torulopsis Berlese Emend.
(Torulopsis Berlese p.p.)

(2) Also placed here . . . . . Kloeckera Janke

e. No true mycelium, no pseudomycelium, no fermentation . . . . .
Cryptococcoideae emend.

(1) Globose or ovoid cells, no starch synthesis . . . . . .
Paratorulopsis N. & Z.
(Torulopsis Berlese p.p.)

(2) Starch synthesis . . . . . Cryptococcus Kütz. emend. Vuill.

(3) Globose or ovoid black cells. Nigrococcus N. & Z.
Rhodotorula Harrison
Dioszegia Zsolt
Trigonopsis Schnacher
Pityrosporum Sabouraud

V. Seccion de Bioquimica, Instituto Nacional de Investigaciones Agronomicas, Ciudad Universitaria, Madrid (3). Communicated by Dr. Santa Maria.

As this is our first communication to the News Letter, we shall list the papers on yeasts issued since 1957:

J. Santa Maria. Formation by Sacch. cerevisiae of asci with more than four spores. J. Bact. 74, 692, 1957.

" Sobre especies pluriesporuladas del genero Saccharomyces. Bol. Inst. Inv. Agronomicas 37, 231, 1957.

" Un nuevo genero de levaduras: Citeromyces. Bol. Inst. Inv. Agronomicas 37, 269, 1957.

" Obtencion de un hibrido ilegitimo de Sacch. delbrueckii. Micr. Esp. 10, 451, 1957.

" Poliploidy in Yeasts. Nature 181, 174, 1958.

" Ecologia de las levaduras. I. Nuevas especies aisladas de alpechin. Bol. Inst. Inv. Agronomicas 38, 301, 1958.

" Oxidation of ethyl alcohol to acetic acid by proliferating yeasts. Nature 182, 937, 1958.

" Sporobolomyces marcillae, nov. spec. isolated from the air. Arch. Mikr. 32, 29, 1958.

" Variacion R - S en C. guilliermondii (Cast) Langueron et Guerra. Micr. Esp. 11, 343, 1958.

" Poliploidia en Saccharomyces. An. Inst. Inv. Agronomicas VIII, 3, 679, 1959.

" Oxidacion de alcohol etilico a acido acetico por levaduras vivas. I. Sacch. Aceti nov. spec. y Sacch. Oxidans nov. spec., nuevas especies aisladas de vino. An. Inst. Inv. Agronomicas VIII, 3, 713, 1959.

" Sobre especies pluriesporuladas del genero Saccharomyces. II. Pluriesporulacion en Sacch. fragilis, Sacch. fructuum, Sacch. oviformis, Sacch. oxidans, Sacch. oleaginosus y diversas razas de Sacch. cerevisiae. An. Inst. Inv. Agronomicas VIII, 3, 737, 1959.

" Oxidacion de alcohol etilico a acido acetico por levaduras vivas. II. Caracteristicas de un hibrido entre Sacch. oxidans nov. spec. y Sacch. oleaceus nov. spec. An. Inst. Inv. Agronomicas VIII, 3, 751, 1959.

" Sobre existencia en Saccharomyces de ascosporas de distinto grado de ploidia. An. Inst. Inv. Agronomicas VIII, 3, 761, 1959.

J. Santa Maria. Significado taxonomico de la dehiscencia del asca en las levaduras. An. Inst. Inv. Agronomicas VIII, 3, 773, 1959.

" Ecologia de las levaduras. II. Levaduras del azucar, de la leche condensada y de la remolacha. An. Inst. Inv. Agronomicas VIII, 3, 779, 1959.

" C. atmosphaerica, nov. spec., aislada del aire. An. Inst. Inv. Agronomicas VIII, 3, 797, 1959.

" Taxonomy of yeasts. Nature 185, 4,715, 781, 1960.

" Saccharomyces hienipiensis: a new melibiose fermenting yeast, unable to assimilate raffinose (to be purlished).

The following two projects on yeasts are now under study:

1. In a natural substratum, the "alpechin" (aqueous solution separated from oil during the manufacture of olive oil), fermentative sporogenous yeasts are easily isolated, among them some cultures which ferment the five sugars: glucose, galactose, sucrose, maltose and melibiose, which represent, undoubtedly, races of Sacch. carlsbergensis. From the possible combinations of these five sugars, 4 by 4, 3 by 3, and 2 by 2 (paying attention that glucose always enters into the combination), and which, respectively, are four, six and four, we find that three combinations of 4, four of 6 and one of 2, appear in the fermentation spectrum in some of the culture isolates up to date. At present we are working with the purpose of obtaining all the mentioned fermentative combinations that are possible by spontaneous or induced mutations of the cultures that ferment the five sugars, and by recombination of those which ferment fewer than five to obtain that which ferments all five. We think that these studies could be useful as a basis for reconsidering the actual conception of species in connection with yeasts.

2. On treating an ascus Le Ac 218 from one of these cultures which are capable of fermenting the above mentioned five sugars, with snail digestive juice (in order to facilitate the separation of ascospores) we have isolated a sporogenous culture, Le Ac 218 A-1 adenine-deficient which produced a pigment, that changes from red to brown red, depending on the medium, and which does not diffuse in the agar. In the absence of adenine it is not able of utilizing ammonium salts, nor aminoacids as nitrogen sources. This culture seems to be very stable. In media without adenine, in which the culture produces pigment, as for example yeast extract-malt extract-peptone-glucose agar, its development is very limited; by adding 0.05 per cent of adenine its development seems to be normal and the pigment disappears.

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

Our project dealing with the yeasts found in the crops of various species of Drosophila, occurring in the Sacramento Valley and in the surrounding hills, is now being completed. Dr. Lidia do Carmo-Sousa, who started the project, has left and she is now studying medical mycology with Dr. Ajello in Atlanta, Georgia. We have also made exploratory studies of yeasts found in Drosophila species occurring at the Pacific sea coast and in a Northern California desert area. Significant differences in yeast flora were found, although some yeasts (such as Sacch. veronae) seem to be ubiquitous.

It has been observed that some species or strains of Sporobolomyces and of Bullera do not discharge or form ballistospores on malt agar and similar rich media, but will do so on corn meal agar. A note, describing this observation, has been accepted for publication in the Journal of Bacteriology.

Four new species of yeast, representing the genera Sporobolomyces, Bullera, Cryptococcus and Candida, have been found in insect infested specimens of Tsuga heterophylla (hemlock). This work will be submitted to Antonie van Leeuwenhoek. Our work (with Mr. Tanaka) on yeast cell wall-digesting enzymes from bacteria is being continued and will be reported in more detail in the Spring issue of the Yeast News Letter.

The following papers have been published:

1. H. J. Phaff and M. Yoneyama, "Endomycopsis scolyti, a new heterothallic species of yeast", Antonie van Leeuwenhoek 27, 196-202, 1961.

2. H. J. Phaff and M. W. Miller, "A specific microflora associated with the fig wasp, Blastophaga psenes Linn. Jour. Insect Pathology 3, 233-243, 1961.

3. Wm. Bridge Cooke, H. J. Phaff, M. W. Miller, M. Shifrine and E. P. Knapp, "Yeasts in polluted water and sewage". Mycologia 52, 210-230 (1960).

4. A. L. Deslisle and H. J. Phaff. "The release of nitrogenous substances by brewer's yeast". Proc. Am. Soc. Brewing Chemists 103-118 (1961).

VII. Institute of Fermentation, Yamanshi University, Kitashimachi, Kofu, Japan. Communicated by Dr. Shoji Goto.

This report is summary of reports at a meeting of the Nippon Nogei Kagakukai, Sept. 21, 1961.

On cheese yeasts; The author studied 14 cheeses from various parts of the world and isolated 31 yeast strains from 5 cheeses. The results of the identification are as follows: Debaryomyces hansenii (9 strains), Deb. subglobosus (6), Deb. kloeckeri (7), Torulopsis sphaerica (2), Trichosporon cutaneum (1), Candida nov. sp. (5) and an unknown strain (1).

This unknown strain is a yeast which forms ascospores and assimilates nitrate. However, this strain differs from Hansenula and Citeromyces by sporulation, absence of fermentation and forms starch-like compounds, etc. This unknown yeast was isolated from Blue cheese.

The cheeses from which yeasts were isolated were Camembert (15 strains), Semi-soft (9), Emmental (3), Blue (3) and Parmesan type (1).

Taxonomic studies of the unknown yeast will be reported in a future paper.

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

Recently Mr. G. Bond and I have tested ten auxotrophs at the adenine-1 locus in Schizosaccharomyces pombe for ability to yield an increased frequency of adenine - independent reversions after treatment with HNO_2 and U.V. Those ten adn-1 mutants had been obtained by Dr. U. Leupold following U.V. irradiation of the wild-type strain.

Our results indicate that only two of the ten adn-1 mutants respond to U.V. treatment. Whether these reversions are due to true back-mutation or to suppressor mutations is as yet unknown. Nitrous acid treatment gave reversions in these two strains which responded to U.V., and also in at least three other mutants which did not respond to U.V. In several cases it was shown that addition of methionine to the plating medium was capable of inhibiting the expression of both spontaneous and induced adn+ reversions.

Recombinational tests were made using large populations of free ascospores, obtained by Leupold's method, from crosses between adn-1 mutants of opposite mating-type. The results indicated that the ten adn-1 mutants used could be arranged linearly at ten different sites within the adn-1 locus. One mutant was peculiar in giving 5-10 times higher frequencies of adenine-independent recombinants, in some crosses, than were obtained normally.

Present work is aimed at analyzing the suppressive effect of methionine on adenine reversions more fully, and the influence of genetic background on mutability. I have recently been able to pay a most useful visit to Dr. H. Heslot in Paris to see something of his work with chemical mutagens on S. pombe.

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

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

1. Papazian, H. P. and Lindegren, C. C. A study of irregular quadruplets in Saccharomyces. Genetics 45: 847-854 (1960).

2. Desborough, Sharon and Shult, Ernest E. Tetrad analysis in Chlamydomonas. Canadian Journal of Genetics and Cytology 3: 325 (1961).

3. Lindegren, C. C. A hypothesis concerning the mechanism of gene-action. Nature 189: 959 (1961).

4. Lindegren, C. C., Lindegren, G., Shult, E. and Hwang, Y. L. Centromeres, sites of affinity and gene loci on the chromosomes of Saccharomyces. Nature. Accepted for publication.

5. Shult, Ernest and Desborough, Sharon, Lindegren, C. C. Preferential segregation in Saccharomyces. Genetical Research. Accepted for publication.

6. Lindegren, C. C. A speculative survey of theoretical genetics. Symposium - New York State Psychiatric Institute.

7. Desborough, Sharon and Shult, Ernest E. An assay for chromosomal and chromatid interference in chromosome V of Saccharomyces. Genetica. Accepted for publication.

X. Dartmouth Medical School, Dept. of Microbiology, Hanover, New Hampshire. Communicated by Dr. S. F. Conti.

I presented a seminar on "Ultrastructure and Function in Yeast" on November 21, at Indiana University. This was part of a lecture series on "Ultrastructure and Function on Microorganisms" being presented at Indiana University under the sponsorship of the Public Health Service. The cordiality and courtesy of the Department of Microbiology, particularly Dr. Thomas O. Brock made this trip both enjoyable and scientifically rewarding.

Our research interests are now focused on the carotenoid pigments of yeast, and investigations of the nuclear cytology of yeast employing an experimental model polarization microscope.

Recent publications on Yeast:

Thyagarajan, T. R., Conti, S. F., and Naylor, H. B. Electron Microscopy of yeast mitochondria, Exp. Cell Res., 25: 216-218, 1961.

Thyagarajan, T. R., Conti, S. F., and Naylor, H. B. Electron Microscopy of Rhodotorula glutinis, J. Bacteriol., in press.

Thyagarajan, T. R., Conti, S. F. and Naylor, H. B. Intranuclear and intracytoplasmic structures of Rhodotorula glutinis as revealed by electron micrographs of serial sections, submitted to J. Biophysic and and Biochem. Cytology.

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

Further investigations have been made on the morphogenesis of C. albicans and the factors controlling morphological transformations in vitro. Formation of "germ tubes" identical to those produced in vivo can be induced in the presence of 5 - 30% human, bovine, rabbit and guinea pig serum. Germ tube formation is completely suppressed when phosphatases are blocked by the addition of 0.1 molar tartrate. No correlation is found between percentage of cells forming germ tubes and pathogenicity towards mice. Fewer germ tubes are found in suckling mice than in adult animals. Germ tubes are not formed in HeLa cells when serum or lactalbumin hydrolysate is omitted from the culture medium.

Work is continuing on the possible occurrence of a phage in yeasts, but so far without the success obtained by Dr. Lindegren. Plaque-like areas have been noted in one colony of C. albicans, but were not reproducible.

The new pigmented yeast reported in Vol. IX No. 2 of the Yeast News Letter contains α and β carotene and is being reported as a Rhodotorula. It is characterized by the presence of a capsule and a marked psychrophilic habit, the optimum temperature apparently being approximately 8°C. Little or no growth occurs above 20°C and exposure to 37°C for 24 hours is lethal.

XII. Department of Biochemistry, University of Birmingham, England. Communicated by Dr. C. Rainbow.

Work by Dr. M. J. Lewis and myself in these laboratories has shown that, during growth of a strain of Saccharomyces cerevisiae (brewer's yeast) on a number of single amino acids as sole major source of nitrogen in a defined medium, transamination with 2-oxoglutarate to yield glutamate is an important early metabolic step. However, cell extracts of the yeast were unable to catalyze transamination between L-arginine and 2-oxoglutarate, nor did they possess arginine desiminase activity, even when prepared from cells grown on L-arginine as sole major source of nitrogen. The utilisation of the nitrogen of L-arginine for growth appeared to proceed by some other pathway which resulted in the formation of proline as end-product, since both the culture liquid after growth of the yeast on L-arginine, and the cell extract prepared from the corresponding cells contained unusually high concentrations of proline.

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

This last summer we completed a comparison of the α-glucosidases produced in response to the five M genes of Winge and several MA genes supplied by Dr. Hawthorne. From studies on heat inactivation, electrophoresis, chromotography on several columns, and neutralization equivalents against antisera as well as substrate specificity we concluded that the enzymes produced in these various genes were identical. Of particular interest was the finding that all of the α-glucosidases were active against sucrose. We have thus far been unable to obtain α-glucosidase (maltase) inactive on sucrose. We would appreciate any observations from people working on yeast who may have seen extracts active on maltose but inactive on sucrose. The finding that several yeast stocks that were unable to ferment sucrose but contained an enzyme active against sucrose further argues for the importance of specific permease systems.

Dr. H. Okada and Mr. John Gorman have succeeded in synthesizing ethylthio-α-D-glucoside. The preparation of this compound has, for the first time, enabled us to examine induction and permeability under conditions of gratuity. They are presently applying this to a study of permeation systems in yeast for α-glucosides. The inducibility by these compounds shows that α-glucoside metabolism is not essential for enzyme induction. Dr. J. Hauge, who has just returned to Oslo, Norway, observed, in studying the kinetics of protein synthesis in yeast, that a fraction rich in lipid and in mitochondrial components was most active in protein synthesis. The isotope was readily incorporated into this fraction and displaced by unlabelled amino acids. Examination in electron micrographs showed the presence of ribosomal-bound particles to membrane components. The identity of this fraction is under present investigation. Dr. Leon Marcus has just joined our group from the University of California, Davis, and will be studying some of the properties of protein synthesis in subcellular fractions.

XIV. Rutgers, The State University, Institute of Microbiology, New Brunswick, New Jersey. Communicated by Dr. J. O. Lampen.

This laboratory has emphasized two programs related to yeast. In one, the effect of the polyene antifungal antibiotics has been investigated, both as a way of determining their mechanism of action and as tools for studying the

nature and functioning of the yeast membrane. For much of this work yeast protoplasts have been employed and from this has evolved the study of the secretion of enzymes, particularly invertase, by the protoplasts.

The following papers either have appeared or will be published before the News Letter issue:

1. Marini, Arnow, and Lampen. J. Gen. Microbiol. 24, 51 (1961). The effect of monovalent cations on the inhibition of yeast metabolism by nystatin.

2. Lampen, In Fungi & Fungus Diseases, Symp. II, N. Y. Acad. of Med., Sect. of Microbiol., Springfield, Ill. Charles C. Thomas. Intermediary metabolism of fungi as revealed by drug action.

3. Lampen. Biochem. Pharmacol. 8, 125 (1961). Changes in yeast permeability induced by nystatin. (Abstract).

4. Lampen and Sutton. Proc. V International Congress of Biochemistry, p. 287 (1961). Synthesis of invertase by yeast protoplasts. (Abstract).

5. Sutton and Lampen. Biochim. et Biophys. Acta, in press, (1961). Localization of sucrose and maltose fermenting systems in Saccharomyces cerevisiae.

6. Sutton, Arnow, and Lampen. Proc. Soc. Exptl. Biol. & Med. 108, 170 (1961). Effect of high concentrations of nystatin upon glycolysis and cellular permeability in yeast.

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

Characteristic aspects on the assimilation and fermentation of sugars by osmophilic yeasts in the presence of high concentrations of sodium chloride were observed.

Assimilation of galactose and of maltose by Saccharomyces rouxii, which is a typical salt-tolerant yeast playing an important role in soy-brewing, was negligible or extremely poor in a medium containing 18% NaCl, although the assimilation in the ordinary medium was vigorous. The yeasts which were able to assimilate and ferment galactose, maltose and/or surcose in the high-saline medium were limited to a few strains.

Sugar assimilability and fermentability are the most important criteria in the taxonomy of yeasts. The present observation, that these properties of the salt-tolerant yeasts can be drastically altered by changing the concentration of NaCl in the medium, should be noteworthy not only in the taxonomy of the osmophilic and halophilic microorganisms, but also in the industrial utilization of these microorganisms.

Reference: H. Onishi, Agr, Biol. Chem. Vol. 25, No. 5, 341 (1961).

Publications:

Studies on Osmophilic yeasts. Part XI. Various factors affecting polyalcohol production by Pichia miso. Agr. Biol. Chem. Vol. 25, No. 2, 124 (1961); Part XIII Conversion of polyalcohol fermentation to ethanol fermentation. Agr. Biol. Chem. Vol. 25, No. 10, 768 (1961).

XVI. Department of Bacteriology, University of Durham, King's College, Newcastle-on-Tyne, England. Communicated by Dr. A. H. Rose.

Since the last edition of Yeast News Letter appeared, I have moved to the Department of Bacteriology in the University of Durham. My two main research projects - the role of biotin in the metabolism of Saccharomyces cerevisiae and the biochemistry of psychrophilic yeasts - are being continued in the new laboratory. Mr. Bernard Dixon has entered upon a period of study for his doctorate; he is working on the role of biotin in membrane production by yeast. Fazal Ahmad has been awarded the degree of Ph.D. (University of Edinburgh) for a thesis entitled "Chemical Studies on biotin-deficient Yeast". He is moving to the Department of Microbiology in the Hahnemann Medical College in Philadelphia to work with Dr. A. G. Moat. Dr. P-O. Hagen also has submitted a thesis - "Studies on the Biochemistry of a Psychrophilic Cryptococcus". Mr. Hagen is to take up a fellowship in the National Research Council of Canada in Ottawa, working with Dr. N. E. Gibbons on psychrophilic microorganisms.

The following publications have appeared during the past year:

A. H. Rose. Industrial Microbiology. Butterworths. 284 pp. 1961.

P-O. Hagen & A. H. Rose. A Psychrophilic Cryptococcus. Canad. J. Microbiol. 7, 287 (1961).

J. L. Dunwell, Fazal Ahmad & A. H. Rose. Changes in the Polysaccharide Composition of Yeast Resulting from Biotin Deficiency. Biochim. biophys. Acta 52, 604 (1961).

A. H. Rose. New Penicillins. Scientific American 204, 66 (1961).

XVII. Department of Avian Medicine, University of California, Davis. Communicated by Dr. M. Shifrine.

Recently Dr. Miller and I have published the following paper: "Classifying yeasts on punch cards", Antonie van Leeuwenhoek 27, 189-192 (1961). I am interested in any comments yeast taxonomists may have about such a system.

Work with Dr. A. G. Marr is in progress on the nutrition of Pityrosporum ovale. Oleic acid is not required for growth of this yeast, palmitic acid is. Myristic and palmitic acids have been found, by gas chromatography, to be contaminants in commercial oleic acid, labelled C.P. A paper on this subject will be submitted shortly to the Journal of General Microbiology.

Dr. Phaff and I are studying the nutritional requirements of certain species of Prototheca isolated from slime fluxes. In a minimal medium we find an absolute requirement for glutamic acid; thiamine is stimulatory.

The exosporium of Saccharomycopsis guttulatus is being studied in thin sections with the electron microscope.

I would appreciate receiving cultures of Pityrosporum arbiculare Gordon.

XVIII. Dr. Rolph Siepman (present address: Chemisches Institute, University of Bonn, West Germany) presents a summary of work done in Bremerhaven together with Dr. Höhnk. The full article is to appear soon in the Bremerhaven Journal.

Summary

During two voyages on the North Atlantic Ocean, 120 yeasts and 9 fungus cultures were isolated from fish, shrimp eggs, sea cucumbers, etc. The following species were identified: Hansenula californica, Debaryomyces kloeckeri, Deb. subglobosus, Cryptococcus laurentii, Cr. albidus, Torulopsis candida, Trichosporon cutaneum, Tr. maritinum nov. sp., Tr. atlanticum nov. sp., Tr. piscium n. sp., Rh. rubra, Rh. mucilaginosa, Rh. glutinis, Rh. texensis, Pullularia pullulans, Fusarium conglutinans, Fus. solanii.

Deb. subglobosus was isolated particularly often. The strong riboflavin excretion by many of these isolates during the assimilation tests points to the possibility that D. subglobosus acts in the capacity of furnishing vitamins to e.g. sponges and sea cucumbers, from which part of these cultures were obtained. It was necessary to observe the sugar assimilation tests (done on a shaker according to the method of Ahearn et al.) for at least 140 days. Yeasts which contained adaptive enzymes (e.g. lactase in Deb. subglobosus) started to grow often after a long starvation period (up to 17 weeks) before utilizing the carbon source. This was also observed with sucrose in the case of H. californica, Tr. cutaneum and Tr. piscium. Gene mutations may be involved in these cases. The majority of the yeasts isolated lacked the ability to ferment.

XIX. Miscellaneous News Items

1. On Oct. 1, 1960 the Mycological Society of Japan held a Symposium on the physiology, cytology and phylogeny of yeasts at Osaka University with the cooperation of the Yeast Discussion Group of Japan.

On that occasion, I proposed and discussed "The origin of the anascosporogenous yeasts". A paper on this problem is now in press in Transactions of the Mycological Society of Japan.

I am now engaged in monographic studies on the genera Endomyces and Endomycopsis. These are very difficult genera, because of the fact that so many species have been imperfectly described without details of asci and physiological characters.

Dr. Yosio Kobayasi
National Science Museum
Ueno Park, Daito-ku, Tokyo, Japan

2. Since it was established in 1944, the Institute for Fermentation, Osaka has rendered service for Japanese and foreign microbiological fields as a culture collection center of fungi and bacteria. Recently, there was a change of the staff organization in the institute. The function of Director was taken over by Dr. Takezi Hasegawa, and in the mycological section, Dr. Keisuke Tubaki has joined us as a new member transferring from Nagao Institute.

A new issue of the culture list (the third edition) is now prepared and will be completed in January, 1962.

Dr. T. Hasegawa
Inst. for Fermentation, Jusonishinocho, 4-54
Higashiyodogawaku, Osaka, Japan

3. Dr. R. C. von Borstel, Oak Ridge National Laboratory, P. O. Box Y, Oakridge, Tennessee, reports the following:

A small genetics research conference was held November 16 to 18 in Carbondale, Illinois. The conference was sponsored by the Committee on Maintenance of Genetic Stocks of the Genetics Society of America. The purpose of the meeting was to discuss exchange of tester strains and to standardize the nomenclature and enumeration of genetic markers in yeast. Those present were R. J. Doyle, S. Fogel, D. C. Hawthorne, C. C. Lindegren, G. Lindegren, G. E. Magni, R. K. Mortimer, H. Ogur, D. Pittman, E. E. Shult, Yuh Lin Hwang, and R. C. von Borstel. A complete report of the meeting is being prepared and it will be printed in the next issue of the Yeast News Letter.

4. Dr. Cyril Rainbow, who until September 30, 1961 was a Senior Lecturer in the Department of Biochemistry, the University of Birmingham, England, has been appointed Chief Chemist, Bass, Ratcliff and Gretton Ltd., Burton-on-Trent, England.

5. Dr. J. Kleyn, Sicks Rainier Brewing Company, Seattle 4, Wash. writes:

Our laboratory is buisly engaged in various projects related to the isolation and selection of better strains of Brewers Yeast as well as writing up some past research related to H_2S production by brewers yeast. We are interested in obtaining new brewers yeast strains and would be happy to exchange cultures with other interested parties.

6. Prof. J. Boidin, Laboratoire de Microbiologie et Mycologie, 16, quai Claude Bernard, Lyon 7 (Rhone), France writes:

Two of my students are now working on the genus Candida. We would like to receive the type cultures and publications containing descriptions of new species of Candida, published during the last few years.

7. During July and August I was able to make some very pleasant visits to a number of yeast workers in Europe and the United States. Beginning in Copenhagen I saw Dr. C. Roberts, formerly of the Carlsberg Laboratories, and Dr. A. Lund of the Tuborg Breweries, and then went to Delft where I spent a day with Miss W. Sloof of the Centraalbureau voor Schimmelcultures. In England I met Dr. J. Barnett of the Low Temperature Research Station at Cambridge, and in the United States Dr. S. Hutner of the Haskins Laboratories, New York; Dr. M. Silva of the Dermatology Department, College of Physicians and Surgeons, Columbia University; Professor S. P. Meyer, Dr. F. Roth, Miss S. A. Meyers and Messrs. D. Ahearne and J. Fell, all of the University of Miami; Dr. L. J. Wickerham of the Northern Utilization Research Branch, Dept. of Agriculture, Peoria, and Professor H. J. Phaff and Dr. M. Miller of the University of California. Also at Davis I was pleased to meet Dr. L. do Carmo Sousa of the Botanical Institute at Lisbon, a laboratory which I had not had the opportunity of visiting.

After Davis I leave for Hawaii where I want to collect some soil samples. Results from New Zealand suggest the two chief factors affecting soil yeast populations, both quantitatively and qualitatively, are moisture and temperature; it will be interesting to see whether this obtains outside New Zealand. One of the most enlightening as well as enjoyable things in my tour has been looking at substrates from which other workers have isolated yeasts and seeing how much environment can affect them. This, together with what are apparently only minor differences in sampling and isolation techniques often reconciles what look to be major differences in reported yeast populations from similar habitats.

Margaret di Menna
Soil Bureau Experiment Station
Lower Hutt, New Zealand

8. Publications from the Mycology Section, N.I.H., have included a group of papers on a new antibiotic, X-5079C, which is effective against some of the mycoses, although not against Cryptococcus and other yeast-like pathogens.

Recently, I gave the annual dinner lecture of the Washington Branch of the American Society for Microbiology.

I was in Japan for ten days last month where I gave lectures on medical mycology and visited laboratories in four cities of Japan.

Dr. Chester W. Emmons, Chief
Medical Mycology Section
National Institute of Allergy and
Infectious Diseases
Bethesda 14, Maryland

9. I have seen recently a remarkably good film on the cytology of yeasts ("Cytomorphologie der Hefen"), made at the Institut für Mikrobiologie und Experimentelle Therapie, Jena, by Dr. Rudolf Müller. The film describes the way yeast cells bud or split. There are pictures of the processes in Sacch. cerevisiae, Schizosaccharomyces, Saccharomycodes, Pichia, Sacch. fragilis, Endomycopsis vernalis, E. capsularis, Sacch. carlsbergensis and a species of Candida. A great deal of cytological detail can be seen, including the behavior of the cell wall, vacuoles, mitochondrial granules and alleged nuclear apparatus.

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

10. Studies with fat soluble stilbyl triazole compounds have indicated that flocculent class III yeasts adsorb the compound and fluoresce much more than do the class I non-flocculent cells.

R. M. Lycette and L. R. Hedrick, 1961 - Use of Fat Soluble Fluorescent Brighteners on Microorganisms. Science 134, 1415.

Physical factors affecting adsorption and kinetics of absorption will be published later.

L. R. Hedrick
Illinois Institute of Technology
Chicago 16, Illinois

11. At the First Annual Meeting of the New York Association of Medical Mycology, New York City, September 22, 1961, Dr. Mercedes R. Edwards gave a lecture on the Electron Microscopy of Pathogenic Yeasts.

Details on the cell structure of Histoplasma capsulatum, Blastomyces dermatitidis and B. brasiliensis were discussed.

Mercedes R. Edwards, Ph.D.
Division of Laboratories and Research
New York State Department of Health
Albany, New York

12. Since 1944, the University of California has received specimens of dead and diseased insects for purposes of diagnosis. This service is provided without charge to any individual or institution anywhere in the world. The Department of Insect Pathology is in a position to attempt the diagnosis of any type of infectious disease (bacterial, fungus, protozoan, virus, etc.) of any insect or arachnid. We are particularly anxious and hopeful of obtaining insects suffering from frank yeast infections. There have been very few reports of insects suffering from diseases caused by yeasts, although examples such as Monocenella and Mycoderma have been reported. For further details and instructions for submitting specimens, write to:

Department of Insect Pathology
University of California
Berkeley, California
Dr. Edward A. Steinhaus, Chairman

13. I am collecting records of where Drosophila spp. (the vinegar flies) have been a nuisance in the wine, brewing, and distilling industries. The nuisance can be by transmission of unwanted yeasts, by gross invasion of the vats or premises, or by the larvae breeding in the raw materials.

There must be many such records in journals to which I do not have access, and I would welcome any references from any part of the world.

E. B. Basden
Institute of Animal Genetics
West Mains Road
Edinburgh 9, Scotland

14. A paper by R. C. Artagaveytia-Allende "Tables for easy identification of the yeasts" was published by U.N.E.S.C.O. (Center of Scientific Cooperation for Latin America).

The organization of these tables is primarily based on the biochemical properties of the yeasts described by Lodder and Kreger van Rij.

A review on the yeasts described after 1952 is now in press. The review on the non-sporulating species will be published first. Later, another on the sporulating species will come out.

A comparative study of the biochemical properties of the yeasts accepted by Lodder and Kreger van Rij is now being done.

The ecological studies on the yeasts isolated from nature are being continued.

C. R. Cano-Marotta and D. Brancho de Kalamar (Fermentation Division) continue working on the ecology of the yeasts isolated from grapes, musts and wines from Uruguay. The results for two different areas will be published soon.

C. R. Cano-Marotta is studying the physiology of the species belonging to Saccharomyces. Some useful physiological tests were added to the current ones.

Any information on the description of new species would be highly appreciated.

Dr. Sanchez Marroquin (Faculty of Chemistry, University of Mexico) was invited to offer here a course on "Industrial Microbiology". The course was given in May and was very interesting.

R. C. Artagaveytia-Allende
D. Fermentaciones Y Enologia
Laboratorio de Micologia
Facultad de Quimica
Montevideo, Uruguay

15. Miscellaneous publications:

Studies on the Nutrition of Thermophilic Yeasts - Torulopsis pintolopesii, Candida slooffii, Candida bovina and Saccharomyces tellustris. Amadeu Cury, Elma N. Suassuna, L. R. Travassos, Anais de Microbiologia, 1960, Vol. 8, Institute de Microbiologia, Av. Pasteur 250, Rio de Janeiro, Brazil.

A Permanganate-chrome Fixative and Lead Acetate Staining for Electron Microscopy of Microorganisms. Noboru Kawakami, Journal of Electronmicroscopy Vol. 10, No. 1, 1961. Department of Fermentation Technology, Faculty of Engineering, Hiroshima University, Hiroshima, Japan.

Polysaccharide-Protein Complexes of Yeast Cell Walls. Walter J. Nickerson, G. Falcone, and Gian Kessler, Macromolecular Complexes, Society of General Physiologists, 6th Annual Symposium Publication, 1961. Institute of Microbiology, Rutgers; New Brunswick, New Jersey.

Letters to the Editor

Dear Sir,

I would like to take this opportunity to present some opinions on a series of recently published papers dealing with the ultrastructure of yeast. Although these views could just as well be expressed in publications dealing with yeast ultrastructure, it is felt that such publications should be concerned primarily with experimental results, with just a minimal amount of space devoted to appraisal of other investigations in the field. The publications in question (Mundkur, B. D., Exptl. Cell Res., 20, 28, 1960); 21, 201, 1960; 25, 1, 1961; and 25, 24, 1961 contain such an abundance of misstatements of fact, erroneous conclusions and artifacts, that the time should be taken to point out that this situation does exist. Since a detailed analysis of these publications would be too bulky, time-consuming, and out of place, it is suggested for those interested that the following publications be read prior to any evaluation of the views and micrographs by Dr. Mundkur.

Hirano, T. and Lindegren, C. C. J. of Ultrastructural Res., 5, 321 (1961).

Vitols, E., Horne, R. J., and Linnane, A. W. J. Biophysic. and Biochem. Cytol., 9, 689 (1961).

Robinow, C. F. J. Biophysic. and Biochem. Cytol. 9, 879 (1961).

Yotsuyunagi, Y., Compt. Rend. 248, 274 (1959).

ibid. 250, 1522 (1960).

Agar, H. and Douglas, H. C., J. Bacteriol. 73, 365 (1957).

Since our publications necessarily reflect our point of view and certain results obtained with our own particular techniques, these should be consulted only cursorily.

For those who have neither the time nor desire to read these papers, it is suggested that some of the results presented by Dr. Mundkur (Exptl. Cell Res. 25, No. 1, pp 1-40) be compared to those obtained by Kawakami, N. (page 179) and Thyagarajan, et al. (page 216) which appear in the same issue. It is felt that even a brief perusal of this issue will illustrate that however valuable a tool freeze drying and staining procedures may prove to be in the study of yeast ultrastructure, the advocation of these techniques to the exclusion of others is questionable.

Samuel F. Conti, Ph.D.
Assistant Professor
Dartmouth Medical School
Hanover, New Hampshire

Dear Sir,

Novak, E. K. and J. Zsolt, 1961. A new system proposed for yeasts. Acta Botanica Hungaricae 7 (1-2): 93-145, with 51 tables.

In line with present tendencies in mycological classification, this system is not a particularly surprising development. Neither is it particularly Friesian in concept. There are a few departures from the classic system as found in Lodder and Kreger-van Rij. Two are especially evident: 1. the inclusion of the imperfect groups in the Endomycetales; and 2. a heavier reliance on such characters as fermentation and mycelium at the generic levels.

The most objectionable feature of the publication is the detail of the mechanics of nomenclature. According to the International Code of Botanical Nomenclature a genus is typified by a species, and that type species is inseparably associated with that genus forever more. A subfamily, family or suborder is typified by, and named in accordance with, the type genus of the subfamily, family, etc., in question. The authors establish within the Saccharomycetaceae, emended, four subfamilies, three of which are not established in this way but on the basis of morphological characteristics on which they are based. It will be noted that the genus Saccharomyces is placed in the subfamily Laevigatosporoideae. This, of course, should be the Saccharomycetoideae.

Where a genus is split into two or four, there is no indication which one contains the type of the original species unless in one of the tables one familiar with the typification of each genus can sort out the type species. However, in the case of Candida, now divided into 4 genera pairs of which form two new subfamilies, C. albicans is included now in Procandida, a situation which cannot stand up under the Rules. Where synonymy is cited, the complete reference is not given either in the system or the tables. This, too, is in violation of the Rules, and is almost Lloydian in concept.

The 51 tables list for each of the 51 genera all the 284 species recognized by late 1959 (the end of the bibliography) according to 26 categories found in the Delft scheme. None of the extra carbon sources of the Wickerham scheme are included in these tables.

Accompanying this letter is a synopsis of the families, subfamilies and genera recognized or established in this paper. These are based on the authors' theory that the following stages are fundamental in the evolution of yeasts:

filament → filament + budding cells → budding cells → budding cells + pseudomycelium

filament → filament + arthrospores → arthrospores

haplobiosis → haplo-diplobiosis → diplobiosis

glucose utilization only → utilization also of other sugars with adaptive enzymes → utilization also of other sugars with constitutive enzymes

respiration → respiration ⟩ fermentation → respiration ⟨ fermentation

One can see that a lot of taxonomic and nomenclatural skull dudgery is necessary to bring both the categories cited by Verona and Montemartini, and those used in Novak and Zsolt's work, which are out of line, into line with the Rules. Further, it may be suggested that in using Wickerham's 34 carbons additional generic and suprageneric categories may be found with which one may or may not agree but compounding the systematists troubles and making the systematist persona non grata among more practical laboraticians.

William Bridge Cooke
U. S. Public Health Service
Cincinnati 26, Ohio