Y E A S T

A News Letter for Persons Interested in Yeast

May 1965 Volume XIV, Number 1

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Many thanks to those who have contributed to this issue by sending in news items and accounts of research projects. The next issue will be published in November 1965. 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, Julianalaan 67a, Delft, Netherlands. Communicated by Dr. W. Ch. Slooff.

Since the last issue of the Yeast News Letter the following cultures (for which a description has been published) have been received:

Candida cloacae Komagata, Nakase et Katsuya
Kazuo Komagata, T. Nakase and N. Katsuya, J. Gen. Appl. Microbiol. 10: 323, 1964.

Candida fabianii Kodama, Kyono, Iida et Onoyama
K. Kodama, T. Kyono, K. Iida & N. Onoyama, Journal of Fermentation Technology 42: 739, 1964.

Candida maltosa Komagata, Nakase et Katsuya
Kazuo Komagata, T. Nakase and N. Katsuya, J. Gen. Appl. Microbiol. 10: 323, 1964.

Candida parapsilosis (Ashf.) Langeron et Talice var. hokkaii Goto
S. Goto & I. Yokotsuka, Bulletin of the R. Institute of Fermentation Yamanashi University No. 9, p. 86, 1962.

Endomycopsis platypodis Baker et van Rij
J. M. Baker and N. J. W. Kreger-van Rij, Antonie van Leeuwenhoek 30: 433, 1964.

Endomycopsis vini van Rij
N. J. W. Kreger-van Rij, Antonie van Leeuwenhoek 30: 428, 1964.

Pichia delftensis Beech
F. W. Beech, Antonie van Leeuwenhoek 31: 81, 1965.

Pichia etchellsii van Rij
N. J. W. Kreger-van Rij, Antonie van Leeuwenhoek 30: 428, 1964.

Saccharomyces inconspicuus van der Walt
J. P. van der Walt, Antonie van Leeuwenhoek 31: 187, 1965.

Saccharomyces vafer van der Walt
J. P. van der Walt, Antonie van Leeuwenhoek 31: 187, 1965.

Trichosporon penicillatum do Carmo-Sousa
L. do Carmo-Sousa, Antonie van Leeuwenhoek 31: 153, 1965.

II. Université de Lyon, Laboratoire de Biologie Végétale, 43, Boulevard de l'Hippodrome, 43, Villeurbanne (Rhone), France. Communicated by Dr. M. C. Pignal.

Since the last issue of the Yeast News Letter, our laboratory has completed the following articles:

The manuscript "Les Candida sans pouvoir fermentaire" by S. Poncet and M. Arpin has been accepted for publication by Antonie van Leeuwenhoek. A paper by F. Abadie and M. Belmont "L'utilisation par les levures de quelques acides aminés comme sources d'azote et de carbone" has been submitted to the same journal.

The first contribution on "Le genre Pichia sensu lato" has appeared; J. Boidin, M. C. Pignal, Y. Lehodey, A. Vey and F. Abadie, Bull. Soc. Mycol. Fr. 80 (4), 396-438 (1964); a new species Pichia media is described; several new combinations are proposed.

Finally F. Abadie has completed a study on the vitamins necessary for the growth of yeasts.

III. Illinois Institute of Technology, Dept. of Biology, Chicago, Ill. 60616. Communicated by L. R. Hedrick and Marjorie Soyugenc.

A paper on yeasts identified from samples of Lake Michigan bottom mud by L. R. Hedrick and Marjorie Soyugenc was presented by L. R. Hedrick at the eighth conference on Great Lakes Research in March 1965, held at the University of Michigan. This paper has been accepted for publication in the Proceedings of this conference. Comparisons of the frequencies of various species found in mud at different locations of Lake Michigan were made. Of the Hansenula species with saturn-shaped spores, H. saturnus, H. californica, H. beijerinickii and H. suaveolens have been found to date, leaving only H. mrakii yet to be identified from lake mud. Comparisons were also made of the species found in mud to those of water from the same location.

IV. Instituto Nacional de Investigaciones Agronómicas, Sección de Bioquímica, Madrid, Spain. Communicated by Dr. J. Santa María.

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

"Utilization of Saccharose and Maltose by Yeasts", Nature 204, 781, 1964.

"Utilización de sacarosa y maltosa por levaduras", Bol. Inst. Nac. Invest. Agronómicas 50, 1-64, 1964.

"Saccharomyces onubensis nov. spec. aislada de mosto de uva y su relación con Sacch. capensis (synom. Sacch. oviformis)". (Saccharomyces onubensis nov. spec. isolated from grape must and its relation to Sacch. capensis (synom. Sacch. oviformis)), Bol. Inst. Nac. Invest. Agronómicas 51, 527-536, 1964.

The following articles have been accepted for publication:

"Separation of phases with sexually different states in Hansenula californica", J. Bacteriol. 1965.

"Especies del género Prototheca asociadas con el olivo" (Species of the genus Prototheca associated with olive trees), Bol. Inst. Nac. Invest. Agronómicas 1965.

We are now studying a culture of Saccharomyces, which probably is a strain of Sacch. delbrueckii isolated as auxotroph uracil-dependent and its reversion to prototroph type, as well as some topics connected with the characteristics of the genus Pichia.

V. Iowa State University of Science and Technology, Dept. of Bacteriology, Ames, Iowa. Communicated by Dr. Paul A. Hartman.

The following is an Abstract of the Ph.D. Dissertation (1964) of Dr. Harland R. Burmeister, one of my former students.

A STUDY OF THE YEASTS IN ENSILED HIGH MOISTURE CORN

Harland R. Burmeister

(Present address: Northern Regional Research Laboratory, Peoria, Illinois)

The effects of the gaseous environment and temperature on the microbial populations of ensiled high moisture corn were investigated. Molds, coliform bacteria, total aerobic bacteria and yeasts were enumerated at various intervals of ensiling. A total of nearly 1200 yeasts was isolated during these studies. These isolates were identified to determine the species of yeasts associated with corn stored under several environmental conditions.

The number of aerobic bacteria in the ensiled corn increased to about 10^8 per g within a week and remained near that number throughout the storage period. The number of total aerobes was not greatly affected by changes in gas concentrations or by the temperature of storage. Coliform bacteria are of no apparent importance in the deterioration of ensiled high moisture corn. Their numbers were relatively small. Mold numbers were also small, but molds were important in the deterioration of corn at the surface of the silo. A detectable number of molds was usually associated with a high yeast count.

Yeast numbers increased rapidly after an initial aeration series, but a second aeration series caused no pronounced growth response. Microorganisms associated with ensiled high moisture corn apparently required a factor in addition to oxygen for rapid multiplication. The factor limiting the growth of yeasts and bacteria in the ensiling process is, apparently, the lack of an assimilable carbohydrate, even though much starch is present.

The yeast species most frequently isolated from freshly harvested corn was Candida parapsilosis, 73 percent of the isolates, and C. intermedia, 15 percent of the isolates. These two yeasts were isolated infrequently after the third week of storage. Yeasts isolated after the twelfth day of storage were principally of the species Hansenula anomala, 66 percent, and C. krusei, 26 percent.

The preponderance of H. anomala and C. krusei in ensiled high moisture corn is believed to be associated with the availability of particular carbohydrates. A study of carbohydrates assimilated by the yeasts isolated from ensiled corn was made. Hansenula anomala, C. krusei and C. tropicalis were the only species of yeasts of those isolated from the corn able to assimilate lactic acid. Lactic acid bacteria have been shown to be the predominant bacterial flora of ensiled high moisture corn. The conversion of sugars to lactic acid by these bacteria, apparently, provides a medium that restricts the growth of a majority of the yeasts placed into the silos along with the corn.

VI. Lehrstuhl für Mikrobiologie und mikrobiologische Abteilung im Institut für Gärungsgewerbe, Seestr. 13, 1 Berlin 65 (West). Communicated by Dr. S. Windisch.

Studies on maltose fermentation in Saccharomyces have been continued. In a wild strain of S. carlsbergensis we found a new gene for maltose fermentation, M 7 (H. Oeser and S. Windisch: Naturwiss. 51, 122, 1964).

Since my last contribution to the YNL the following articles have been published:

1) S. Windisch and W. Bronn: Stand und Leistung der technischen Mikrobiologie. Zbl.f.Bakt. I Orig. 191, 149-159, 1963.

2) S. Windisch: Moderne Methoden der biologischen Betriebskontrolle in der Brauerei. Monatsschrift für Brauerei 18, 8-11, 1965.

3) S. Windisch: Methoden, Leistungsfähigkeit und Beurteilung von mikrobiologischen Luftanalysen. Monatsschrift für Brauerei 18, 96-104, 1965.

4) S. Windisch and I. Neumann: Zur mikrobiologischen Untersuchung von Marzipan. 1. Mitteilung: Keimzahl und Keime der Rohmassen. 2. Mitteilung: Vergleichende Untersuchung der Keimzahl und besonders des Gehalts an Hefen von Rohmasse, Zucker und Marzipan. Süsswaren 9, 355-358 und 484-490, 1965.

The last two articles concern a new topic: the microbiology of "Marzipan", a kind of sweets which is made industrially from almonds and sugar. The yeast flora of Marzipan has been investigated in order to learn if there are yeasts which cause deterioration. Only strains of Saccharomyces rouxii have been found to be harmful to the production of Marzipan. The research work on S. rouxii and its technical importance will be continued.

In another paper two new species of yeast-like fungi are described. One species, Protendomycopsis domschii n.g.n.sp., is a typical representative of the Saccharomycetaceae, as its diploid cells turn into asci. The other species, Endomyces laibachii n.sp., has an interesting life cycle. It forms a mycelium which disarticulates. The free arthrospores act as ascus mother cells. They become one-, two- or four-spored asci. Neither species ferments any sugars, but are ready to assimilate a great number of substances as single carbon source. Both species seem to be different perfect stages of Trichosporon cutaneum auctorum. Latin diagnoses of the new or little-known taxa are added. The taxonomic relations of the Endomycetaceae, Saccharomycetaceae and of the genera concerned are discussed. (Beiträge zur Biologie der Pflanzen 1965, in press.)

Dr. C. C. Emeis has become Privatdozent for microbiology. He adds a report upon his latest papers:

C. C. Emeis: The amount of pitching yeast and the age of cells.

The age distribution of cells in yeast populations under different conditions was studied. The age of the single cells was estimated by counting the bud-scars on the surface of their walls. It was observed that under various conditions the age distribution is a constant proportion, 50% of the cells have no bud-scars, 25% only one scar, 12.5% two scars, 6.25% three scars and so on. Experiments to vary this constant proportion in proliferating yeast populations by changing the environmental conditions had no success. (Monatsschrift für Brauerei, in press.)

C. C. Emeis: Biological aspects of rapid fermentations ("Schnellgärung").

The cell concentration is an important factor in rapid and continuous fermentations. Experiments with different amounts of pitching yeast showed

that the increase of yeast concentration in the wort is a function of the dissolved oxygen only. The absolute increase in yeast concentration is independent of the amount of pitching yeast in a range up to the four-fold. At 25°C the yeast autolysizes in a few days. It is, therefore, very important to separate the yeast from the fermented wort directly after fermentation has finished. (Monatsschrift für Brauerei, in press.)

Privatdozent Dr. H. Gutz, who was with me since 1955 as scientific assistant, has accepted a position as Assistant Professor in the Division of Genetics, Lab. of Molec. Sciences of the South West Center for Advanced Studies in Dallas, Texas.

VII. Dept. of Food Science and Technology, University of California, Davis, Calif. 95616. Communicated by Dr. H. J. Phaff.

1. The following is an abstract of an article appearing in the Ciferri Memorial Issue of Rivista di Patologia vegetale, Serie III, Vol. IV, 485-497 (1964):

A ONE-YEAR, QUANTITATIVE STUDY OF THE YEAST FLORA IN A SINGLE SLIME FLUX OF ULMUS CARPINIFOLIA GLED.

H. J. Phaff, M. Yoneyama and Lidia do Carmo-Sousa

ABSTRACT

A quantitative study was made of the yeast flora of a single slime flux of the smooth-leaved elm, Ulmus carpinifolia, during an annual cycle. Pichia pastoris was found in all samples and was quantitatively the most abundant species. Also present in all samples, but in much lower numbers, was Trichosporon penicillatum. A third major organism was Prototheca moriformis, which was more abundant than Tr. penicillatum during the summer, but appeared virtually absent during the winter months. Prototheca ciferri and Pr. zopfii occurred more rarely. Occasionally other species of yeast formed a small part of the total population of the above-mentioned major types. Evidence is presented that these species were probably introduced by visiting insects.

2. Enzymatic Hydrolysis of Yeast Cell Walls. I. Isolation of wall-decomposing organisms and purification of lytic enzymes. H. Tanaka and H. J. Phaff. To appear in the June 1965 issue of the Journal of Bacteriology.

ABSTRACT

A number of microorganisms, able to decompose and grow on yeast cell walls, was isolated from soil. These isolates demonstrated various types of attack on yeast walls. A bacterium, identified as Bacillus circulans, and a species of Streptomyces produced clear, lysed zones when grown on an agar medium containing baker's yeast cell walls. The streptomycete formed glucanase, mannanase and protease, but B. circulans produced only glucanases. Purified mannan could be prepared from the culture fluid of B. circulans grown on baker's yeast cell walls. In a liquid, mineral medium, extracellular lytic enzyme production by B. circulans was optimal after 3 days of aerobic growth at 30°C with 0.5% baker's yeast cell walls as the carbon source. Twelve other carbon sources were ineffective as inducers. Among a number of polysaccharides tested the crude enzymes of B. circulans hydrolyzed only β-1→3 glucan (laminarin) and β-1→6 glucan (pustulan), both by a random mechanism, to a mixture of dimer and glucose. The β-1→3 and β-1→6 glucanases

were separated from each other by DEAE cellulose column chromatography. Water-soluble oat glucan, which contains in the linear chain both β-1→3 and β-1→4 bonds, was also hydrolyzed by the bacterial β-1→3 glucanase. The products of this reaction indicated that this enzyme also hydrolyzes β-1→4 glucosidic linkages, provided the β-glucopyranosyl unit composing this bond is substituted in the 3-position by another glucose unit.

3. Professor H. J. Phaff will participate in the international Symposium on Yeast-Protoplasts to be held September 21-24 in Jena, G.D.R. He will present a paper (to be published jointly with Dr. H. Tanaka) entitled "Action of bacterial β-glucanases on isolated cell walls and ascus walls of various yeasts."

VIII. Department of Zoology, University of Edinburgh, Scotland. Communicated by Professor J. M. Mitchison.

Summary from "Preparation of Synchronous Cultures by Sedimentation" by J. M. Mitchison and W. S. Vincent. Nature, 205, 987, 1965.

Synchronous cultures of budding yeast, fission yeast, or E. coli can be made by centrifuging a layer of packed cells through a linear sucrose gradient. The smaller cells, at the beginning of the cell cycle, move more slowly through the gradient and can be separated off and grown as a synchronous culture. The technique is simple and will produce a 200 ml synchronous culture from one gradient tube.

We are now using this method for investigating various biochemical events in the cell cycle of Schizosaccharomyces pombe - RNA and DNA synthesis, enzyme activity, and the pool kinetics of RNA precursors.

Summary from "Selective synthesis of messenger RNA in a fission yeast during a step-down, and its relation to the cell cycle" by J. M. Mitchison and P. R. Gross. Exp. Cell Res. 37, 259, 1965.

Cells of Schizosaccharomyces pombe are transferred from a rich medium to a minimal medium containing ethanol. For a time after this "step-down", it appears that the RNA which is synthesized is largely messenger RNA because of its instability, its base ratios, and its sedimentation properties.

We are completing the second half of this work which is an autoradiographic study of cells labelled with RNA precursors after a step-down.

IX. Department of Botany, University of Dacca, Pakistan. Communicated by Professor Majeed Ahmad, Head of the Department of Botany.

Studies on an interesting Saccharomyces carlsbergensis mutant:

I: Interference by methyl group in the utilization of pyrimidines.

While studying the genetics of a local baker's yeast, Haradhan (1962) observed a spontaneous mutant arising from a single-spore culture. He could not determine its nutritional deficiency. It has now been established to be a double mutant. It responds to a mixture of lysine and uracil or lysine and cytosine, showing thereby its capacity to interconvert uracil and cytosine.

This interconversion of uracil and cytosine was also accomplished by two more uracil-less yeast mutants.

The double mutant failed to grow in lysine plus thymine, indicating that it is not a general pyrimidine mutant. Since thymine differs from uracil and cytosine in having a $-CH_3$ group at the 5th carbon atom, it was suspected that probably the methyl group in thymine interferes with its utilization. The lack of utilization of 5-methyl cytosine by this mutant confirmed that the methyl group interferes with the utilization of pyrimidines by the mutant.

Preliminary genetic studies of the mutant have shown that its defective lysine and cytosine loci are probably linked.

Recent publication:

Ahmad, M. 1964. Incompatibility in Yeast. In Symposium on Incompatibility in Fungi. (In press).

Trips taken:

Professor Majeed Ahmad participated in the International Congress on "Science Teaching and Economic Growth" held at Dakar (Senegal), January 14-22, 1965.

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

Dr. A. Herman has previously isolated and mapped four U.V. induced histidine requiring mutants of Saccharomyces lactis. Three of these loci were found to be linked. The fourth locus mapped close to the centromere-linked mating locus on a separate linkage group. The three linked loci govern amido transferase, isomerase and IGP dehydrase activities (hi-7, hi-6, hi-3 respectively), while the unlinked locus controlled histindinal dehydrogenase activity. A comparison of the genetic maps showed that there was little correlation between S. lactis and non-interbreeding Lindegren Breeding Stock, since in the latter material hi-7, hi-6 and hi-3 are on separate linkage groups. The only parallel which can be drawn is that in both S. lactis and Lindegren Breeding Stock the hi-4 gene and mating locus are closely linked on one linkage group.

A marked increase in the rate and extent of the transfer of amino acids from aminoacyl-sRNA to proteins has been achieved by looking at conditions of maximal stability of the substrate (pH 6.5, ammonium maleate buffer). GDP is a strong inhibitor of the reaction by competing competitively with GTP, the affinities of the system for both nucleotides being of the same order of magnitude. The lack of requirement for GTP and of inhibition by GDP of the binding of phenylalanine-sRNA to the ribosomes, points to the participation of GTP in the polymerization step.

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

The following articles have been published since the last issue of the Yeast News Letter:

Bhattacharjee, J. K. and Lindegren, G. Gene control of pigmentation associated with a specific lysine requirement of Saccharomyces. Biophysical Research Communications 17: 554 (1964).

Hwang, Donna S. and Lindegren, Carl C. The inductions of an alpha-glucosidase by glucose. Nature 205: 880 (1965).

Hwang, Donna S., Lindegren, C. C., Bhattacharjee, J. K. and Roshanmanesh, A. The genetic integrity of upgraded and downgraded alleles of the melezitose locus in Saccharomyces. Canadian Journal of Genetics and Cytology 6: 414-418 (1964).

Lindegren, Carl C. C. P. Snow, Optimist. A review of The Two Cultures: and a Second Look by C. P. Snow. The Humanish #6, November-December (1964), page 193.

Lindegren, Carl C., Bang, Y. N. and Osumi, Masako. The central body of the ascomycetes. Canadian Journal of Genetics and Cytology 7: 37-39 (1965).

Lindegren, G., Oshima, Y., Hwang, Y. L. and Lindegren, C. C. Chromosomal rearrangement in Saccharomyces induced by ethyl methanesulfonate. Abstract, American Society for Microbiology, Bacteriological Proceedings 1965. Atlantic City, New Jersey, April 25-29 (1965) page 24.

XII. Institute "G. Marañon", 138 Velarquez, Madrid 6, Spain. Communicated by Dr. A. Sols.

It has been found that growth of yeast at the expense of non-sugar carbon sources involves an inducible fructosediphosphatase. This enzyme by-passes the physiologically irreversible phosphofructokinase reaction in the glyconeogenesis required for polysaccharide synthesis in these conditions. The fructosediphosphatase of Sac. cerevisiae is highly specific (Km ca. 2×10^{-7} M) and is inhibited allosterically by AMP (Ki 8×10^{-5} M). Appearance of the fructosediphosphatase is accompanied by a decrease in the amount of the antagonistic phosphofructokinase.

(C. Gancedo, M. L. Salas, A. Giner and A. Sols, "Reciprocal effects of carbon sources on the levels of an AMP-sensitive fructose-1,6-diphosphatase and phosphofructokinase in yeast". Biochem. Biophys. Res. Commun., in press).

XIII. Komensky University, Department of Biochemistry, Bratislava, Sasinkova 5/II, Czechoslovakia. Communicated by Dr. Ladislav Kovač.

The problems of coupling of catabolic and anabolic processes in the cell are under study in this Laboratory. It has been shown recently that the inhibitors of oxidative phosphorylation, dinitrophenol and azide, inhibit anabolic reactions anaerobically in anaerobically-grown yeast, i.e. in cells lacking the complete system of oxidative phosphorylation. The results suggest a role of partial reactions of oxidative phosphorylation in coupling of catabolism and anabolism. This is supported by the finding that anaerobically grown ergosterol- and unsaturated fatty acid-deficient yeast is unable of this coupling, despite the fact that normal catabolic and anabolic reactions can be demonstrated in vitro; a role of structural lipids in the coupling is implicated. The properties of these nutritionally lipid-deficient yeasts are studied in relation to fermentative and oxidative systems and an attempt is made to prepare yeast mutants auxotrophic for lipids.

XIV. Rutgers - The State University, Institute of Microbiology, New Brunswick, New Jersey 08903. Communicated by Dr. J. O. Lampen, Director.

In our laboratory, work is continuing on the secretion of invertase and on the detailed structure of the protein and its relation to the yeast

cell wall. Also, purification of the enzymes for protoplasting our strains of yeast is in progress.

A review on Secretion of enzymes by micro-organisms was presented in April 1965 at the Symposium of the Society for General Microbiology on Function and Structure in Micro-organisms. The secretion of invertase by yeast protoplasts and of amylase and penicillinase by bacilli were used to illustrate the general problem. The presentation was primarily an attempt to present a consistent working hypothesis, and it is hoped that comments will be received from others interested in the problem of the mechanism of enzyme secretion.

XV. Falstaff Brewing Corporation, Bioengineering Department, St. Louis, Missouri. Communicated by Dr. C. Akin.

The following are abstracts of papers presented at the American Society of Brewing Chemists, May 9-14, 1965 and the American Society for Microbiology, April 25-29, 1965 conventions.

KINETICS OF YEAST SEDIMENTATION IN AQUEOUS SUSPENSIONS

Cavit Akin and Suzanne Lagomarcino

This paper will be published in the 1965 A.S.B.C. conventions proceedings.

A study was conducted to determine the effects of pH and temperature on the sedimentation rate of Saccharomyces cerevisiae (Falstaff strain 76). The sedimentation apparatus was a glass tube 1.5 cm diameter and 120 cm height, enclosed in a glass cooling jacket. The yeast concentration was 3×10^8 cells/ml suspended in phthalate buffers from pH 2.3 to 5.9.

The experiments indicated that sedimentation occurs in four phases: Flocculation, settling, packing and clarification. A critical pH was observed between 3.6 and 3.9. The sedimentation rate was nearly zero cm/min for pH 2.4, 3.2 and 3.6, while for pH's 3.95, 4.65 and 5.65 the rates were 7.0, 6.9 and 7.1 cm/min.

A series of tests were conducted at temperatures 3, 7, 10 and 15°C and a constant pH of 4.15. As the temperature increases, the settling rate increases. However, there is an inverse relationship between temperature and rate of flocculation. Below 7°C, the yeast rapidly agglutinated into large flocs. Smaller flocs formed above 7°C.

THE REMOVAL OF DIACETYL IN BEER BY SACCHAROMYCES CEREVISIAE

Suzanne Lagomarcino and Cavit Akin

We studied the temperature, diacetyl concentration, and yeast concentration in order to determine the effects of these fermentation variables on the removal rate and pattern of diacetyl. Known amounts of diacetyl and Saccharomyces cerevisiae (Falstaff strain #76) were added to degassed beer in magnetically stirred flasks. Samples, taken at zero, two, four, six and twenty-four hours, were determined for diacetyl by the vicinal diketone method. Temperature studies were conducted between 3 and 20°C. A yeast concentration of 1.5 g cake per liter was used. The initial diacetyl concentration was 1.5 ppm.

The temperature studies indicated that at temperatures below 13°C, the diacetyl removal rates were slow and decreased slightly as the temperature decreased. The instantaneous removal rates at the 6th hour for 16 and 20°C were

approximately 0.05 ppm diacetyl per hour compared to 0.03 ppm diacetyl per hour at 13°C. Initial diacetyl concentrations ranging from 0.37 ppm to 1.5 ppm were tested at 16°C. The lowest initial diacetyl concentration had the lowest rate of removal; 0.01 as compared to 0.05 ppm diacetyl/hr for 1.5 ppm diacetyl.

The yeast concentration studies illustrated that the higher the concentration, the higher the removal rate. During the first four hours of the test, the average removal rates were 0.045, 0.11, 0.15, and 0.18 ppm diacetyl per hour for 0.75, 1.5, 6 and 12 g yeast per liter.

XVI. Industrial Fermentations Section, Microbiological Department, Egyptian Ministry of Agriculture, University Street, Giza, U.A.R. Communicated by Dr. A. S. El-Nawawy and M. A. Fouda.

In April 1965, the first Conference for Applied Microbiology was held at Cairo. Among the papers read at the Conference, the following were concerned with yeasts:

1) Isolation and selection of yeast strains highly efficient in growth on pentose substrates. By A. S. El-Nawawy, Y. Abdelmalek, and M. Fahmy.

Two strains, isolated from fermenting figs and straw compost, proved to be highly efficient in utilizing hydrolysates of corn cobs and stalks. Comparing them with a strain of Candida utilis, they produced higher yields and more protein. The two strains were identified as Candida pelliculosa, an organism that has not been tested before for industrial uses.

2) Optimum conditions for the production of fodder yeast (Candida pelliculosa), grown on maize cobs hydrolysate. By A. S. El-Nawawy, Y. Abdelmalek, and M. Fahmy.

Best yield of C. pelliculosa was obtained under the following conditions: a) Corn cobs hydrolysate diluted to 12.5 g/l of reducing sugar (as xylose), and containing not more than 100 mg/l of furfural was most satisfactory. When the hydrolysate was steam distilled (furfural removal) the concentration of reducing sugars could be raised to 15 g/l. b) Propagation time was 44 hrs for maximum protein synthesis and 68 hrs for maximum total yield. c) Continuous aeration at a rate of 15-20 liters of air/hr. d) One gram of yeast was used as a starter for 1 liter of hydrolysate. e) Nitrogen source as $(NH_4)_2SO_4$ added in a concentration of 2.2 g/l, and phosphates as KH_2PO_4 added in a concentration of 0.6 g/l.

The chemical analysis of the yeast produced was: 35% carbohydrates, 43% protein, 4.2% fat, and 7.8% ash. Paper chromatography showed this yeast to contain the following 17 amino acids: glycine, alanine, valine, leucine, isoleucine, methionine, serine, threonine, aspartic acid, glutamic acid, arginine, lycine, tyrosine, tryptophan, histidine, proline, and phenylalanine.

3) Variation in Saccharomyces cerevisiae induced by gamma radiation. By S. M. Taha, S. A. Z. Mahmoud, and M. A. Fouda. Five strains of yeast supplied by the Hawamdia Distilleries (U.A.R.), used for the commercial production of ethanol, were tried for the production of new variants that might be more sugar- and alcohol-tolerant strains, using the irradiation technique. They were identified as Saccharomyces cerevisiae, except for one strain, which was found to be Saccharomyces fructuum. Agar slant cultures, 2 days old, were

irradiated with gamma-rays from Co^{60} and received 25000 r. A simple method for screening was used to select variants that would tolerate higher glucose or ethanol concentrations under both aerobic and anaerobic conditions. The original cultures and the selected variants were tested for glucose and ethanol tolerance; besides, different fermentation abilities towards various sugars, spore-forming abilities, and rates of fermentation were also tested.

Concerning sugar and alcohol tolerance, no significant differences were detected between original and variant cultures. Instead, the rate of fermentation (estimated by Warburg technique for growing cells) was improved in 7 cases out of 9 variants. It increased from 20%-50% in the variants over the corresponding original strains. In the other 2 variants, a slight decrease was detected. The method of screening used was thus successful for obtaining better fermenting yeasts, but not for more tolerating ones.

With regard to the sporulation capacities, all mutants differed from each parent culture. In all cases, improvements either in the total numbers of sporulating cells, in the number of 4-spored asci formed, or in both were confirmed (ex.8% to 27% total asci, and 3% to 37% 4-spored asci).

Tests for fermentation abilities revealed gene mutations concerning the abilities to ferment maltose and galactose. Two different originals which were both maltose (+) and galactose (-); each gave two mutants which became maltose (-) and at the same time galactose (+). The loss of maltose fermenting ability gave rise to the possibility of considering S. cerevisiae and S. fructuum (Lodder & Kreger van Rij, 1952) to be one species, similar to the interpretations of Roberts and van der Walt (1960). The acquired galactose fermenting ability in all derived variants (4 mutants) was found to be the slow fermenting ability requiring an adaptation period. This involved the gene mutation $G^- \rightarrow G_s$.

4) Physiological variation in Saccharomyces cerevisiae attributed to "dikaryosis". By S. M. Taha, S. Z. Mahmoud, and M. A. Fouda.

The microtechniques needed for yeast breeding have been established recently in our laboratories. In the early trials for yeast hybridization using Lindegren's mass mating technique, the resultant diploid structures were incapable of spore formation. When testing these strains for rate of fermentation, it was found that all were, by far, less active fermenters than the diploid parents. The parameter used was CO_2 evolved measured gasometrically by Warburg technique. Polyhydric alcohols (calc. as glycerol) produced by these strains under conditions used for osmophilic yeasts (Haijney, Hendershot and Peterson, 1960) was estimated. Distinct physiological differences between these strains and the parent diploids were once more evident. These results were interpreted on the basis of probable dikaryosis either in the homozygous or heterozygous diploid structures recovered from the hybridization mixture. Among the supporting evidence was the observation of the way of budding of the zygotes. Cytological methods used did not give dependable results.

XVII. Kodama Brewing Co. Ltd., Iizuka, Iidagawa, Showa-Machi, Akita Prefecture, Japan. Communicated by Dr. Kenkichi Kodama.

The following papers have been published:

1. Studies on Wild Yeasts which Thrive in "Sake-moto"; K. Kodama, T. Kyono, K. Iida, and N. Onoyama. Jour. Ferm. Technol. 42, 739-745 (1964).

This paper deals with two species of the genus Candida occasionally isolated from "Koji" which is considered as one of the most prominent sources of wild yeasts which thrive in Shubo "Sake moto".

The samples used for the isolation of yeasts were sent from 22 (Akita pref.) and 32 (Chiba pref.) breweries, respectively. Among these, 66 strains were identified as Candida guilliermondii (Cast.) Langeron et Guerra, which is one of the most widely distributed species. Nine strains (L.K.B.-AK 6, 24, 29, 32, 33, 36, 45, 60, 67) were considered to be a unique species, which assimilates nitrate. Among the known species of Candida, our isolates are similar to Candida pelliculosa Red. However, in malt extract, our isolates do not form any pellicle nor ferment galactose in contrast to C. pelliculosa. Further, in the assimilation of i-erythritol, it is negative for the former, positive for the latter.

Our isolates also resemble Hansenula subpelliculosa Bedford in the lack of pellicle formation and fermentable pattern of sugars. But all our isolates are heterothallic haploid yeasts, in addition they do not assimilate i-erythritol, nor form true mycelium in contrast to H. subpelliculosa.

Therefore, our isolates should be considered as a new species, which the authors wish to name Candida fabianii nov. spec., in honor of the late Dr. Fabian in U.S.A.

Recently Dr. Wickerham found that 4 strains of our isolates (AK-6, 32, 33, and 36) mated with his mating types "undescribed species b". Dr. Wickerham and the authors decided that the former would describe the perfect form as a new species of Hansenula, while the latter would describe the imperfect form, the heterothallic haploid as a new species of Candida mentioned above, in accordance with International Code of Botanical Nomenclature. Two type cultures of the isolates (L.K.B. AK-6 et 32) have been deposited in the Yeast Collection of the Centraalbureau voor Schimmelcultures (Delft, Holland). A Latin diagnosis is given.

2. Prototheca spec. (I) Studies on cultural properties of Prototheca sp.; M. Sakamoto, H. Yamazaki, K. Kodama, and C. Kyono. Jour. Ferm Techmol. 42, 481-485 (1964).

Authors studied cultural properties of Prototheca spec. The following points are noted:

1. Prototheca spec. was found by Kenkichi Kodama in soil from Brazil.

2. Protetheca spec. was of a similar strain when compared with Prototheca zopfii.

3. Patterns of the infrared spectra of Prototheca spec. cells showed a resemblence to Prototheca zopfii and Prototheca trispora.

4. The cell yield of Prototheca spec. was 49.4% against supplied sugar. The authors are further studying the properties and application of Prototheca spec.

3. Debaryomyces nilsonii (Capriotti) nov. comb.; K. Kodama, T. Kyono, H. Naganishi, and Y. Takahara. Jour. Ferm. Technol. 42, 655-660 (1964).

This paper deals with a yeast culture isolated from soil in Brazil. Because of its unique spore morphology (warty wall of the spore), it must doubtlessly be classified in the genus Debaryomyces. In this genus no

species is known which has biochemical characteristics for both fermentation of sugars and assimilation of carbon compounds corresponding to those of our isolate. Further, if the spore morphology is not taken into consideration from the viewpoint of taxonomy, the isolate resembles Torulaspora nilssoni Capriotti in many properties. Particularly, our comparative studies of our isolate with a type culture of T. nilssoni (C. B. S.-2924) revealed that the former agrees quite well with the latter in the characteristics mentioned above. In addition, a distinctly warty wall was observed in the spores of the type culture of the latter under the electron microscope. Therefore, it is of the author's opinion that Torulaspora nilssoni should be transferred to the species of Debaryomyces.

XVIII. Brief News Items.

1. The Editor announces with deep regret the untimely death of Dr. Harry Katznelson on 10 February 1965 at the age of 52. Dr. Katznelson was Director of Canada Department of Agriculture's Microbiology Research Institute and a Fellow of the Royal Society of Canada.

Dr. Katznelson, who had won an international reputation in agricultural microbiology, joined CDA in 1940 at Ottawa as a bacteriologist in the Bacteriology and Dairy Research Division of Science Service. Subsequent promotions led to his appointment as chief of the division and, in 1959, to the post which he held at the time of his death.

The author of more than 120 scientific papers on agricultural microbiology, Dr. Katznelson represented CDA at the Second United Nations International Conference on peaceful uses of atomic energy held in Geneva in 1958. He was also chairman of the microbiology section of the Ninth International Botanical Congress held in Montreal a year later.

Dr. Katznelson was a member and former vice-president of the Canadian Society of Microbiologists. He was also a member of the Agricultural Institute of Canada, the Professional Institute of the Public Service of Canada, the Canadian Phytopathological Society, the International Society of Soil Science and the American Society of Microbiologists.

2. Dr. Esther Meyer (home address: 1617 E. 50th Place, Apt, 1-D, Chicago, Ill., 60615, writes:

I retired January 1965 from my position as associate professor of microbiology, University of Illinois, College of Medicine.

At present I am at home relaxing and enjoying the freedom. Perhaps in the future I shall seek part time employment.

3. Dr. Herbert Gutz, formerly with the Institut für Gaerungsgewerbe of the Technische Universität, Berlin, Germany, has accepted an appointment as Assistant Professor at the Graduate Research Center of the Southwest in Dallas, Texas. In the Division of Genetics of this Center Dr. Gutz will continue his work on yeast genetics. He is especially concerned with the genetics of Schizosaccharomyces pombe, working on problems of gene fine structure, allelic complementation, intragenic recombination and regulation. Dr. Klaus Haefner,

formerly with the Institut für Biophysik of the Freie Universität, Berlin, Germany, is associated with the yeast program. He is working on UV-induced inactivation and mutation in polyploid strains of Saccharomyces.

4. Dr. Jørgen Friis writes: I have changed address and position recently. I have spent the last three years in the Department of Genetics, University of Washington, Seattle and have now a position at the Department of Genetics, University of Copenhagen, and space for my laboratory has kindly been provided by the Carlsberg Laboratory, Department of Physiology.

I would like you to tell readers of the News Letter that I would be very happy to receive reprints of work dealing with yeasts, taxonomy, biochemistry, physiology and genetics. At the same time I would be very glad to meet scientists with an interest in yeast, if they have a chance to come to Copenhagen. My new address is: Jørgen Friis, Carlsberg Laboratory, Physiological Department, 1o, Gl. Carlsbergvej, Copenhagen, Valby, Denmark. Telephone: VA. 2455.

5. Dr. L. J. Wickerham (Northern Regional Research Laboratory, U.S.D.A., Peoria, Illinois, 61604) writes: Dr. Frank H. Stodola, Head of the Pioneering Laboratory of the Northern Regional Research Laboratory, has received the Annual Pasteur Award on May 22 from the Illinois Society for Microbiology. Dr. Stodola has done structural studies on the sphingolipides produced by Hansenula ciferrii (a series of four papers), and he is senior author on a paper to appear in BIOCHEMISTRY on "8,9,13-Triacetoxydocosanoic Acid, an Extracellular Lipid Produced by a Yeast."

I am currently writing papers on new species of heterothallic haploid species of Hansenula. Two will appear in the Ciferri memorial issue of MYCOPATHOLOGIA ET MYCOLOGIA APPLICATA.

6. Dr. Colin H. Clarke (Institute of Animal Genetics, West Mains Rd., Edinburgh, Scotland) writes: I am at present spending one year working in Professor R. P. Wagner's department at the University of Texas, Austin, learning something of valine biosynthesis by mitochondrially-associated enzymes in Neurospora crassa.

The following papers have been accepted for publication:

N. Loprieno and C. H. Clarke. Investigations on reversions to methionine-independence by mutagens in Schizosaccharomyces pombe. Mutation Research (in press).

Anwar Nasim and C. H. Clarke. Nitrous acid-induced mosaicism in Schizosaccharomyces pombe. Mutation Research (in press).

C. H. Clarke. Methionine as an antimutagen in Schizosaccharomyces pombe. J. Gen. Microbiol. (in press).

7. Dr. A. C. Blackwood, Dept. of Microbiology, MacDonald College of McGill University, Que., Canada, writes: One of my students, Mr. R. E. Simard, has presented a thesis as partial requirement for an M.Sc. degree entitled: "Characteristics of Yeasts Isolated from Various Ecological Sources." This thesis is available if requests are made through the Library, MacDonald College.

8. Dr. S. H. Hutner (Haskins Laboratories, 305 East 43rd Street, New York, N. Y., 10017) has supplied the following news item: Luis R. Travassos of the Institute of Microbiology of the University of Brazil, 250 Avenida Pasteur, Rio de Janeiro, is preparing a dissertation on methyl group metabolism in the enteric yeast Candida slooffii. It is expected that this material will be submitted for publication by the end of the year.

The work on enteric yeasts has been pushed steadily by my colleagues in the Institute of Microbiology at Rio de Janeiro.

9. Dr. R. C. Hatfield, California State Polytechnic College, San Luis Obispo, Calif., writes: I have a new book, just published, APPLIED MICROBIOLOGY, National Press, Palo Alto (1965), which contains several experiments on yeats--growth curves, wine yeast isolation, etc.

We are starting a research project on the effect of certain metallic ions on yeasts.

10. The following publications have been received recently from Dr. J. J. Miller, Dept. of Biology, McMaster University, Hamilton, Ontario, Canada.

J. J. Miller and O. Hoffmann-Ostenhof. Spore formation and germination in Saccharomyces. Zeitschrift für Allg. Mikrobiologie 4, 273-294 (1964).

J. J. Miller. A comparison of the effects of several nutrients and inhibitors on yeast meiosis and mitosis. Experimental Cell Res. 33, 46-49 (1964).

C. Ramirez and J. J. Miller. The metabolism of yeast sporulation. VI. Changes in amino acid content during sporogenesis. Canadian Jour. Microbiol. 10, 623-631 (1964).

11. Arthur Guinness Son & Co. (Park Royal) Ltd., Park Royal, London, N.W. 10, England. Dr. John S. Pierce has sent reprints of the following recently published papers.

P. J. Mill. The nature of the interactions between flocculent cells in the flocculation of Saccharomyces cerevisiae. J. gen. Microbiol. 35, 61-68 (1964).

V. E. Chester. Comparative studies on the dissimilation of reserve carbohydrate in four strains of Saccharomyces cerevisiae. Biochem. J. 92, 318-323 (1964).

Margaret Jones and J. S. Pierce. Absorption of amino acids from wort by yeasts. Journal of the Institute of Brewing 70, 307-315 (1964).

12. Professor O. Verona, Director, Institute of Plant Pathology and Agricultural Microbiology of University of Pisa (Italy) has sent the following two publications:

G. Picci and O. Verona. Sulla flora blastomycetica di alcuni animali marini. La ricerca scientifica, Rediconti B. Vol. 4, 85-96, 1964.

Summary

On the blastomycetic flora of some marine animals.

Numerous strains of yeast isolated from fishes and other marine animals from the northern part of the Thyrrenian were determined.

Sporogenous yeasts were infrequent; they were Saccharomyces and mainly Debaryomyces species. Asporogenous yeasts were more common and almost exclusively Candida species. Within this genus, C. parapsilosis and C. guilliermondii species and their varieties predominated

Also published:

O. Verona, G. Picci, A. Lepidi, and G. Mazzucchetti. Sopra alcuni lieviti presenti nella pasta-legno e sulla attivita cellulosolitica di alcuni Trichosporon. L'Agricoltura Italiana, Jan. Feb. 1965, p. 1-12.