Y E A S T

A News Letter for Persons Interested in Yeast

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The next issue will be assembled towards the end of November. Although a reminder for copy will be mailed in the fall, news items may be sent in at any time.

H. J. Phaff

I Centraalbureau voor Schimmelcultures, Delft, Julianalaan 67a. Communicated by Dr. D. Yarrow.

Below follows a list of new species in the collection of the Yeast Division.

Candida bombi CBS 5836
Candida obtusa var. arabinosa CBS 5837
Candida oleophila CBS 2219
Candida santamariae CBS 4515
Candida santamariae var. membranaefaciens CBS 5838
R. Montrocher, Rev. Mycol. 32, 69, 1967.
Candida edax CBS 5657
J. P. van der Walt and E. E. Nel, A. v. Leeuwenhoek, 34, 106, 1968.
Candida suecica CBS 5724
L. Rodrigues de Miranda and B. Norkrans, A. v. Leeuwenhoek, 34, 115, 1968.
Pichia angophorae CBS 5823
M. W. Miller and E. R. Barker, A. v. Leeuwenhoek, 34, 183, 1968.
Saccharomyces carmosousae CBS 5901
R. Montrocher, Bull. Soc. Mycol. France, 83, 641, 1967.
Zygolipomyces lactosus CBS 5911
Zygolipomyces tetrasporus CBS 5910
N. P. Krassilnikov, I. P. Babjeva and K. Meavahd, Mikrobiologiya 36, 923, 1967.

An article by R. Scheda and D. Yarrow entitled "Variation in the fermentative pattern of some Saccharomyces species" will be published in Archiv für Mikrobiologie very soon.

II Laboratoire de Biologie Végétale, Université de Lyon, Villeurbanne, France. Communicated by Dr. Melle M. C. Pignal.

Since the last issue of the Yeast News Letter the following articles have appeared:

- J. B. FIOL Intérêt systematique des tests de croissance en milieu déficient en vitamines pour les genres Kluyveromyces et Pichia. Revue de Mycologie 32: 45-56 1967.
- R. MONTROCHER Quelques nouvelles espèces et variétés du genre Candida (Levures asporogènes). Revue de Mycologie 32:69-92 1967.

A description has been given of a new species, Candida bombi, and of two new varieties, C. santamariae var. membranaefaciens and C. obtusa var. arabinosa; besides, two varieties have been elevated to the rank of species and one species was lowered to the rank of variety. The type cultures have been deposited in the CBS culture collection.

- R. MONTROCHER Les Candida à pouvoir fermentaire et n'assimilant pas les nitrates. Bull. Soc. Mycol. Fr. 83:641-730 1967.

A detailed comparative study of the morphological and physiological properties of 41 species of Candida, able to ferment but not able to assimilate nitrate, enabled the author to establish a certain number of groups. A correlation between this attempt of grouping and that based on serology is discussed. A key for species determination of this category of Candidas is given.

III Department of yeasts of the Tsechoslovakian Collection of Microorganisms in the Institute of Chemistry of the Slovak Academy of Sciences, Bratislava, Dúbravska cesta. Communicated by Dr. Anna Kocková-Kratochvílová.

1. The following papers appeared up to the end of 1967:

Kocková-Kratochvílová, A., Šandula, J., Vojtková-Lepšíková, A., Pokorná, M., Stuchlík, V.: The genus Candida Berkhout. VIII. Fermentation type II species. Folia microbiologica 12:327-344, 1967.

Sedlárová, L.: Diploidisierungsfähigkeit und ihre Zusammenfassung mit Sexualitätsproblemen bei Hefen. Biológia 22:831-838, 1967.

Pokorná, M., Kocková-Kratochvílová, A., Stuchlík, V., Hronská, L.: The genus Candida Berkhout. IX. Continuous cultivation of Candida albicans. Folia microbiologica 12:447-457, 1967.

2. The following papers will appear during 1968.

Kocková-Kratochvílová, A.: Einige probleme der numerischen Taxonomie bei der Hefen. Mitteilungen der Versuchstation für die Gärungsgewerbe, Wien, 22:Nr. 5/6, 1968.

The Genus Saccharomyces (Meyen) Reess. V. Species Saccharomyces willianus Saccardo and related species. Folia microbiologica 13:Nr. 5, 1968.

Appendix to the above mentioned paper./Kocková-Kratochvílová, A./, Folia microbiologica 13:Nr. 5, 1968.

Kocková-Kratochvílová, A., Šandula, J., Vojtková-Lepšíková, A.: The Genus Candida Berkhout. X. Candida parapsilosis (Ashford) Langeron et Guerra. Folia microbiologica, in press.

Vojtková-Lepšíková, A., Kocková-Kratochvílová, A.: Amylolytic enzymes in Candida tropicalis. Biológia, in press.

Kocková-Kratochvílová, A., Šandula, J., Vojtková-Lepšíková, A., Sedlárová, L., Kasmanová, M.: The taxometric study of the genus Saccharomyces (Meyen) Reess. First part: Species completely fermenting raffinose. Edition: "Biologické práce" (English) in press: Publish. of the Slov. Acad. Sci. Bratislava.

A new Catalogue of microorganisms of the Tsechoslovakian Collections will appear in Brno, July 1968.

3. The following lecture was delivered for the assembly of the II. International Symposium of fermentation industry, Leipzig (DDR), May 27, 1968:

Kocková-Kratochvílová A.: Die Bedeutung der taxonomischen Forschung der Gattung Saccharomyces für die Gärungsindustrie.

4. Three dissertations have been completed recently under the direction of Dr. A. Kocková-Kratochvílová:

T. Stryčková-Petrovová: Biochemical and cytological studies of cell wall polysaccharides of Candida guilliermondii.

J. Leško: Glycoproteins isolated from horse and beef serum.

K. Tomášek: The quality of the Slovak hop.

5. The following manuscripts will be completed during 1968:

Kocková-Kratochvílová A., Sedlárová L., Vojtková-Lepšíková A., Šándula J.: The taxometric study of the genus Saccharomyces (Meyen) Reess. Second part: Saccharomyces cerevisiae Hansen and related species. Edition: "Biologické práce" (English). Publ. by the Slov. Acad. Sci. Bratislava.

Kocková-Kratochvílová A., Sedlárová L.: The genus Saccharomyces (Meyen) Reess. VII. A taxometric study of strains of various ploidy. (English).

IV Ajinomoto Co., Inc., Central Research Laboratories, 2964, Suzuki-cho, Kawasaki, Japan. Communicated by Dr. T. Nakase.

The following paper will be presented at the International Conference on Culture Collections which will be held in Tokyo, from October 7-12, 1968.

TAXONOMIC SIGNIFICANCE OF BASE COMPOSITION OF YEAST DNA
T. Nakase and K. Komagata

The base composition of DNA (GC content) from 140 species of yeasts and yeast-like fungi was studied, and a taxonomic significance of this character was found. The GC contents were widely distributed from 26 to 65.5%. Species in the Saccharomycetaceae exhibited low GC contents, namely, 26 to 47.5%. Naganishia globosus, the only urease positive species in this family, demonstrated the highest value. Intrageneric variation was 10.5% in Endomycopsis, 12% in Saccharomyces, 13% in Debaryomyces, 16% in Pichia, and 14.5% in Hansenula. No significant generic difference was found in this family.

In the Cryptococcaceae, the GC content ranged from 28 to 65.5%. Yeasts belonging to Cryptococcus and Rhodotorula exhibited high GC contents, 46 to 56% and 47.5 to 65.5%, respectively. In the genera Torulopsis, Candida and Trichosporon, GC contents were widely distributed, namely, 33.5 to 58% in Torulopsis, 28 to 60% in Candida. and 32.5 to 59% in Trichosporon. This fact indicates the heterogeneity of these genera.

Of the yeast-like fungi, Tremella fuciformis and Aureobasidium pullulans exhibited high GC contents; meanwhile, Dipodascus albidus and Geotrichum candidum showed low GC values comparable to those of the Saccharomycetaceae.

Species which exhibited strong urease activity demonstrated high GC contents (46 to 65.5%) with no exceptions. Species of Cryptococcus, Rhodotorula and Sporobolomyces were included in this group. Further, the same fact was found in some species of Torulopsis, Candida and Trichosporon. These yeasts are supposed to be related to the Heterobasidiomycetes. From these facts, the GC content and urease activity are considered to be good taxonomic criteria.

The GC contents given in the paper are as follows:

Yeasts	GC Content (mole %)	Yeasts	GC Content (mole %)
Saccharomyces		Pichia (Continued)	
S. kamakuraensis	42.5	P. fluxuum	30
S. rosei	40	P. chambardii	28
S. florentinus	38.5	P. kluyveri	26-27
S. cerevisiae	36-36.5		
S. lactis	36	Hansenula	
S. delbrueckii	32	H. angusta	45
S. exiguus	30.5	H. minuta	43
		H. capsulata	42.5
Endomycopsis		H. fabianii	42.5
E. capsulata	40	H. saturnus	39.5
E. fibuligera	37.5	H. canadensis	35.5
E. fasciculata	35.5	H. beckii	34
E. muscicola	34.5	H. schneggii	33.5
E. platypodis	33.5	H. anomala	32.5-33.5
E. javanensis	29.5	H. ciferrii	30.5
Pichia		Debaryomyces	
Pichia sp.	42	D. franciscae	42
P. wickerhamii	41.5	D. marama	34.5
P. pinus	41	D. hansenii	34.5
P. ohmeri	40.5-41.5	D. cantarellii	30
P. fermentans	40	D. vanriji	29
P. membranaefaciens	40		
P. pastoris	39.5	Schwanniomyces occidentalis	31.5
P. pijperi	39.5	Hanseniaspora valbyensis	30
P. bovis	38.5	Lipomyces starkeyi	45.5
P. minuscula	38	Kluyveromyces polysporus	31
P. farinosa	37-38	Citeromyces matritensis	42.5
P. salictaria	36.5	Pachysolen tannophilus	40
P. haplophila	36.5	Wickerhamia fluorescens	35
P. orientalis	36	Naganishia globosus	47.5
P. etchellsii	35.5		
P. zaruensis	35	Cryptococcus	
P. vini	34.5	Cr. laurentii	56
P. vini var. melibiosi	34.5	Cr. terreus	52.5
P. terricola	34	Cr. diffluens	51

Yeasts	GC Content (mole %)
Cryptococcus (Continued)	
Cr. neoformans var. uniguttulatus	48.5
Cr. neoformans	46
Torulopsis	
T. magnoliae	58
T. ingeniosa	53
T. aeria	52.5
T. torresii	48.5
T. colliculosa	40.5
T. stellata	40.5
T. sphaerica	36.5
T. glabrata	35.5
T. pinus	34.5
T. ernobii	34
Torulopsis sp.	33.5
Candida	
C. humicola	60
C. diffluens	57
C. curvata	56
C. scottii	55.5
C. bogoriensis	53
C. gelida	52
C. parapsilosis var. hokkai	52
C. zeylanoides	51.5
C. punicea	51
C. catenulata	49.5
C. frigida	49.5
C. japonica	47.5
C. rugosa	47
C. pulcherrima	44.5
C. incommunis	42
C. vinaria	41.5
C. guilliermondii	41
C. intermedia	41
C. monosa	40
C. tenuis	40
C. utilis	40
C. glaebosa	39.5
C. lusitaniae	39
C. krusei var. transitoria	39
C. fimetaria	38.5
C. krusei	38
C. sorbosa	38
C. parapsilosis	37.5
C. parapsilosis var. querci	37.5
C. macedoniensis	37
Candida (Continued)	
C. pseudotropicalis	37
C. melinii	36.5
C. salmonicola	36.5
C. robusta	36
C. cloacae	35.5
C. maltosa	33.5
C. natalensis	33.5
C. trigonopsoides	33
C. claussenii	33
C. albicans	32.5
C. norvegenesis	32.5
C. langeronii	32.5
C. tropicalis	32
C. krusei var. saccharicola	32
C. stellatoidea	32
C. benhamii	31
C. koshuensis	30
C. citrea	29.5
C. polymorpha	29
C. pseudosorbosa	28.5
C. rugopellicula	28
Rhodotorula	
R. infirmo-miniata	65.5
R. glutinis	64
R. rubra	63.5
R. lactosa	54
R. peneaus	51
R. marina	50
R. pallida	49.5
R. flava	48
R. zsoltii	48
R. texensis	47.5-48
R. slooffii	47.5
Trichosporon	
Trich. pullulans	54
Trich. cutaneum	59
Trich. behrendii	32.5
Brettanomyces bruxellensis	35
Trigonopsis variabilis	44
Kloeckera apiculata	30
Sporobolomyces salmonicolor	57
Aureobasidium pullulans	51.5
Dipodascus albidus	33
Geotrichum candidum	40.5
Tremella fuciformis	54.5

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

The following paper has been accepted for publication in the Biochemical Journal: "Exo-β-glucanases in Yeast", by Ahmed T. H. Abd-El-Al and H. J. Phaff.

Miss Sally A. Meyer has completed the requirements for the M. A. degree in Microbiology under the guidance of Professor H. J. Phaff.

A summary of her thesis "Isolation and Base Composition of DNA from Yeasts" is given below.

Fifteen species of yeasts were examined for their DNA base composition (See Table). This study included (1) developing a satisfactory method of cell disruption and DNA isolation, (2) determining base composition by the melting temperature procedure, and (3) evaluating the taxonomic significance of the resultant base compositions.

Method

A simple and convenient method was developed for the isolation of high molecular weight DNA from yeasts. Yeasts were grown in large batches and lyophilized. The dried yeast was suspended (25% solids) in saline-EDTA (0.1M), sodium dodecyl sulfate (2%), and mercaptoethanol (1%) and then exposed to chloroform vapors at 37C overnight. The resulting viscous suspension was treated according to the method of Marmur for the isolation and purification of DNA. A few modifications were necessary to adapt the method for yeast cells. These included (1) several treatments with ribonuclease to remove the large amounts of RNA, (2) the addition of phenol in the deproteinization step immediately following the treatment with RNase and (3) dialysis of the final product against standard saline citrate solution.

Taxonomic significance of base compositions in yeast:

1. Two strains of different geographic origin of the species, Lodderomyces elongasporus, revealed the same GC content.

2. The proposed asexual form, Candida parapsilosis, of L. elongasporus revealed the same GC content.

3. Saccharomyces rosei and the physiologically related species, S. bisporus and S. inconspicuus, had GC contents which were approximately 7-9% higher than that of S. cerevisiae.

4. Debaryomyces globosus showed a GC content more aligned to the S. rosei group of Saccharomyces than to D. hansenii.

5. The recently proposed relationship of C. atmosphaerica to C. diddensii is supported by their similar GC content.

6. The Candida species show a large range in GC content demonstrating the heterogeneity of the genus.

7. Realization of the limitations of examining only one strain of a species is obvious. For any taxonomic evaluation several strains of the same species should be investigated.

DNA base composition in yeasts

Organism	T_m* with standard deviation	GC %**
Candida tropicalis	83.6 ± 0.28	34.9
Debaryomyces hansenii	84.3 ± 0.26	36.6
Saccharomyces cerevisiae	84.5 ± 0.13	37.1
Candida diddensii	85.6 ± 0.22	39.8
Lodderomyces elongasporus	85.5 ± 0.27	39.5
Lodderomyces elongasporus***	85.6 ± 0.08	39.8
Candida parapsilosis	85.7 ± 0.24	40.0
Candida atmosphaerica	86.2 ± 0.17	41.2
Chlamydozyma reukaufii	86.7 ± 0.17	42.2
Saccharomyces rosei	87.3 ± 0.29	43.9
Saccharomyces bisporus	87.5 ± 0.09	44.4
Metschnikowia bicuspidata	87.6 ± 0.05	44.6
Chlamydozyma pulcherrima	87.6 ± 0.13	44.6
Debaryomyces globosus	87.8 ± 0.29	45.1
Saccharomyces inconspicuus	88.3 ± 0.22	46.3
Candida oregonensis	89.0 ± 0.03	48.0
Calf thymus****	86.7 ± 0.10	42.4

*Average of at least three independent determinations for yeast species.

**Increasing order.

***Type strain.

****Control sample of DNA.

The following is an abstract of a recently completed doctoral dissertation by Dr. Edward J. Buecher, Jr. under the guidance of Professor H. J. Phaff.

Physiology, Dimorphism, and Cell Wall Biochemistry of *Saccharomycopsis* Schionning

Saccharomycopsis guttulata, a yeast which inhabits the gastrointestinal tract of rabbits, was investigated. Twelve strains were isolated from domestic New Zealand white rabbits (100% incidence) and 2 strains from wild black-tailed jack rabbits (50% incidence). *S. guttulata* was not observed or isolated from the stomach contents or feces of golden mantled ground squirrels, belding squirrels, California ground squirrels, and California grey squirrels. All strains assimilated glucose, sucrose, raffinose, and inulin (latently) and fermented glucose and sucrose. All strains, except one, were typical budding yeasts. A filamentous strain was isolated from a wild jack rabbit.

Continuous gassing of liquid cultures showed that all budding strains required 10-15% CO_2 for optimal growth and that they grew at the same rate between 0.25% and 20% O_2. Under optimal gas conditions exponential growth occurred, resulting in high yields of healthy cells. With sufficient CO_2 growth also occurred on several media previously reported to be unsuitable and at a temperature as low as 30°, whereas up to now the minimal temperature of growth was thought to be 35°. Single cells developed in liquid media supplied with CO_2, whereas in static cultures they die before sufficient metabolic CO_2 develops to support growth.

The filamentous strain (OV-2) differed from all other strains in that: (i) it existed in both a yeast and a filamentous form; (ii) the filamentous form, in contrast to the budding form, grew in the absence of CO_2 at 37°, but at 30° the filamentous form also required CO_2; (iii) the growth rates of both forms at 37° were approximately one-half the growth rate of the normal budding strains; (iv) the filamentous form may produce buds under certain conditions.

Modification of the gas phase helped to clarify the conditions favoring the formation of buds from filaments. This morphogenetic change exhibited itself in the form of constrictions at the hyphal tips and was induced by strictly anaerobic conditions; this is followed by active end budding only in the presence of a little oxygen (e.g. 2%) and 10-15% CO_2. Isolated single cells produced by end budding required CO_2 and remained in the budding yeast form, producing an occasional filament, presumably by mutation.

The chemical composition of purified walls of various strains grown under different environmental conditions was determined. Strains were similar in their wall composition, except that the filamentous form had slightly higher chitin, phosphate, and mannose contents and a lower protein content. Values for the various components of *S. guttulata* wall preparations were 70-74.3% total carbohydrate (glucan and mannan only, in an approximate ratio of 2:1), 14.8-21.8% total protein, 1.6-2.3% chitin and 0.04-0.09% total phosphate. Comparable concentrations of the amino acids aspartic acid, serine, threonine, glutamic acid, proline, glycine, alanine, valine, cystine or cysteic acid, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine were found in the

walls of all strains.

The initial rate of degradation of native S. guttulata walls was much greater with endo-β-1→3 or β-1→6 glucanases than it was with baker's yeast walls. In the presence of 0.02 M 2-mercaptoethanol, or after removal of protein by the proteolytic enzyme Pronase, walls of both yeasts were degraded rapidly by either glucanase. Exhaustive enzymic degradation of the glucan component of the walls showed that S. guttulata contained almost twice as many glucosidic bonds hydrolyzable by β-1→6 glucanase as by β-1→3 glucanase. In Sacch. cerevisiae glucan the numbers of such linkages were about equal. Exhaustive degradation by one glucanase left few attackable linkages for the alternate glucanase in the residual walls; similarly glucans insoluble in boiling 2 N H_2SO_4 (2.5 hrs) of either yeast were not degradable by the two glucanases.

Electron micrographs taken of various native and treated walls of S. guttulata were analyzed and compared with similarly treated walls of Sacch. cerevisiae.

VI Department of Botany and Plant Pathology, Louisiana State University, Baton Rouge, Louisiana. 70803. Communicated by Dr. J. B. Sinclair.

El-Tobshy, Zeineb M., and J. B. Sinclair. 1968. Changes in pathogenicity among three isolates of Geotrichum candidum after serial passage on chorioallantoic membrane of embryonated chicken eggs. Phytopathology 58: (in press). (Abstr.).

"Three isolates of G. candidum, CH (human), ATCC-7019 (citrus), and LA-2 (citrus), were tested for pathogenicity after 10 serial passages on chorioallantoic membranes (CAM) of 10-day-old embryonated chicken eggs at 31C. The window method for virulence assay with arthrospores suspended in normal saline was used. Twenty replications were used for each assay. Data were analyzed using probit analysis for extrapolation of a dosage-mortality curve. LD_{50}, based on arthrospores/embryo, of chick embryos after 3-5 days was computed for the original culture of each isolate and compared with the LD_{50} of the same isolate after serial passage. CH showed a significant increase in virulence with an original LD_{50} at 8,172 and after serial passage, 1,551. ATCC-7019 and a final LD_{50} at 27,572. The difference, however, was not significant. There was no significant difference between the original LD_{50} of LA-2 at 16,479 and final LD_{50} at 10,304. Data indicated a general tendency toward increased parasitic adaptation. No changes in pathogenicity on certain fruits and vegetables could be detected for any isolate after serial passage on CAM."

El-Tobshy, Zeineb M., and J. B. Sinclair. 1968. Histopathology of Geotrichum candidum in the chorioallantoic membrane of embryonated chicken eggs. Phytopathology 58: (in press) (Abstr.).

"Histopathological studies of three isolates of G. candidum: CH (human), ATCC-7019 (citrus), and LA-2 (citrus) on the chorioallantoic membrane (CAM) of embryonated chicken eggs were made using the window method for inoculation. Membranes were inoculated with arthrospore suspensions in normal saline. Membranes were harvested 5-7 days after inoculation and incubation at 31C. The initial reaction of the CAM to infection was hyperplasis with proliferation of the ectoderm where the fungal hyphae had penetrated. Macroscopically, the CAM thickened and showed grayish-yellow to light-brown lesions accompanied by a general deterioration of blood vessels in response to infection by all isolates. Sections of infected membranes fixed in Zenker's solution and stained with hematoxylin and eosin were examined. There was no difference among isolates in manner of growth or in response of the CAM to infection. All isolates grew readily in the CAM. Arthrospores germinated forming hyphae, hyphal fragments, and secondary arthrospores. Hyphal fragments were found in all parts of the CAM including the ectoderm, mesoderm and endoderm."

El-Tobshy, Zeineb M. 1968. Establishment of the pathological relationship between plant and animal isolates of Geotrichum candidum in embryonated chicken eggs. Ph.D. dissertation. Louisiana State University Library, Baton Rouge, Louisiana 70803. 53 p.

"A method of virulence assay of Geotrichum candidum in embryonated chicken eggs was used. Pathogenicity of seven isolates was tested on the chorioallantoic membrane (CAM) of embryonated egg which showed that animal isolates CH and B-446 were more pathogenic than plant isolates LA-2, C-125, WR, ATCC-7019, and LA-1. Yolk sacs were found to be susceptible to isolates CH and ATCC-7019. Susceptibility of embryos through yolk-sac inoculation decreased with age. Yolk sac was more susceptible than the CAM to isolate CH. Other experiments indicated that serial passages of the fungus on the CAM was associated with a tendency for increased pathogenicity. Inoculation of citrus fruit with the isolates after the serial passages showed stability in pathogenicity on plant tissue. Histopathological studies showed that the fungus grew in the CAM, and hyperplasis with proliferation of the tissue followed by disintegration occurred as a response to infection."

VII Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisc. Communicated by Dr. Michael S. Esposito and Dr. Rochelle E. Esposito.

The Genetic Control of Meiosis and Sporulation in Saccharomyces

As part of a study of the biochemical and genetic regulation of meiosis and sporulation in yeast, temperature-sensitive mutants deficient

in their ability to sporulate have been isolated in a homothallic strain of yeast carrying the D (diploidization) gene of Winge and Roberts (Compt. Rend. Lab. Carlsberg Ser. Physiol. 24:341, 1949). To obtain both dominant and recessive mutations of meiosis, haploid ascospores obtained by sporulation of a diploid strain homozygous for the D gene were irradiated with ultraviolet light. The surviving diploid colonies were examined for their ability to sporulate at 20°C, 30°C, and 34°C. By this technique 75 mutants deficient in either meiosis or ascospore development have been obtained. 65/75 of the mutants are temperature sensitive; 16 are sensitive to cold and 49 are sensitive to heat. Several of the mutants when sporulated at a permissive temperature produce a large fraction of inviable ascospores. These mutants may be intimately involved in chromosome segregation and they affect the results of aneuploidy. Genetic recombination and chromosome assortment of mutant strains is currently under investigation. A more detailed account of this work will appear in GENETICS.

VIII Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisc. 53706. Communicated by Professor Harlyn O. Halvorson.

1. Some aspects of the current program of yeast research in our laboratory are summarized below:

SYNTHESIS OF MACROMOLECULES DURING THE CELL CYCLE IN YEAST. Tauro, P., Schweizer, E., Epstein, R., and Halvorson, H. O.

The synthesis of mitochondrial DNA during the cell cycle of S. lactis was investigated. By using equilibrium density gradient centrifugation in $Cs_2SO_4/HgCl_2$ to separate mitochondrial and nuclear DNA the ratio of mitochondrial to total DNA was determined for various periods of the cell cycle. It could be shown, that this ratio varies discontinuously over a narrow interval of the cell cycle, whereas during the remainder of the cycle it remains stable. From these results it is evident that the two DNAs are not synthesized at precisely the same time since in that case the ratio would have remained constant. It is concluded that the synthesis of both nuclear and mitochondrial DNA is discontinuous during the cell cycle and that mitochondrial DNA is synthesized at a time very close to the time of nuclear DNA synthesis.

THE REDUNDANCY OF RIBOSOMAL AND TRANSFER RNA GENES IN SACCHAROMYCES CEREVISIAE. Schweizer, E., MacKecknie, C., and Halvorson, H. O.

DNA-RNA hybridization studies have been performed to determine the number of 4 S, 18 S and 26 S RNA cistrons present in purified nuclear and mitochondrial DNA of S. cerevisiae. It was found that 1% of the nuclear DNA hybridizes with 18 S RNA and 2% with 26 S RNA. Between 0.064 and 0.08% of the nuclear DNA is complementary to transfer RNA. No hybridization was observed between mitochondrial DNA and the three classes of cytoplasmic RNA. Assuming a genome size of yeast nuclear DNA of 2.5×10^{10} daltons the hybridization data correspond to 340 cistrons for 18 S RNA, 340 for 26 S RNA and 320-400 cistrons for transfer RNA. By competition experiments the cistrons for all three classes of RNA can be demonstrated to be distinct and specific.

SPECIFIC HYBRIDIZATION OF MITOCHONDRIAL RNA TO MITOCHONDRIAL DNA. Morimoto, H., Schweizer, E., and Halvorson, H. O.

Mitochondrial RNA prepared from yeast (Saccharomyces cerevisiae) was separated into a fast and a slow sedimenting fraction. Both demonstrate specific hybridization with mitochondrial DNA prepared from the same cells. Mitochondrial RNA fractions exhibit some binding to nuclear DNA but this is competed by cold cytoplasmic ribosomal RNA. Binding of mitochondrial RNA to mitochondrial DNA is competed for by cold mitochondrial RNA, but not by cytoplasmic RNA, indicating that the binding of mitochondrial RNA to mitochondrial DNA is specific. Preliminary studies of the nucleotide composition of mitochondrial RNA indicate that the light and heavy components are of similar composition. The nucleotide content of these fractions is different from that of cytoplasmic ribosomal RNA.

MACROMOLECULAR SYNTHESIS DURING SPORULATION OF YEAST. Esposito, M. S., Esposito, R., and Halvorson, H. O.

The synthesis of RNA, DNA, and protein during sporulation of Saccharomyces has been studied. Cultures sporulating in acetate medium undergo a doubling of DNA beginning at ca 5 hours after introduction into sporulation medium. RNA and protein content increase by ca 40% by 10 hours. The dry weight of sporulating cells shows a similar increase by 12 hours. Asci first appear at 12-14 hours; by 50 hours the populations studied routinely achieve 65% ascus formation. The fate of acetate during sporulation is currently under investigation. Preliminary isotope incorporation studies indicate that approximately 15% of the acetate metabolized becomes involved in macromolecular synthesis.

2. The following recent publications may be of interest to yeast workers:

Marcus, L., Ris, H., Halvorson, H. O., Bretthauer, R. K., and Bock, R. M., 1967. Occurrence, isolation and characterization of polyribosomes in yeast. J. Cell Biol. 34, 505-512.

Marcus, L., and Halvorson, H. O., 1967. Resolution and isolation of yeast polysomes. Methods in Enzymology 12, 498-502.

Nakao, Y., Lee, S. Y., Halvorson, H. O., and Bock, R. M., 1968. The nucleases of yeast. I. Properties and variability of ribonucleases. Biochim. Biophys. Acta 15, 114-125.

Tauro, P., Halvorson, H. O., and Epstein, R. L., 1968. Time of gene expression in relation to centromere distance during the cell cycle of Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. 59, pp. 277-284.

Tingle, M., Herman, A., Halvorson, H. O. Characterization and Mapping of Histidine Genes in Saccharomyces lactis. Genetics 58, pp. 361-371. March 1968.

IX Dept. of Bacteriology, Indiana University, Bloomington, Indiana 47405. Communicated by Dr. M. A. Crandall and Dr. T. D. Brock.

This summary is of a recently completed Ph.D. dissertation "Genetic and Biochemical Studies of Sexual Agglutination in the Yeast Hansenula wingei" (Crandall, M. A., Indiana University, Bloomington, Indiana, 1968).

Various aspects of this work, as well as work by T. D. Brock, have been accepted for publication in "Science", "Nature" and "Bacteriological Reviews". The titles of these three papers are respectively: "Molecular Aspects of Specific Cell Contact", "Mutual Repression of Haploid Genes in Diploid Yeast", and "Molecular Basis of Mating in the Yeast Hansenula wingei".

The yeast, Hansenula wingei, was isolated in 1956 by L. J. Wickerham. The two haploid mating types, strains 5 and 21, exhibit a massive sexual agglutination reaction when mixed. This agglutination reaction is due to the interaction of cell surface macromolecules which are complementary. The agglutination factor from strain 5, 5-factor, was isolated by both N. W. Taylor (1964) and T. D. Brock (1965). 5-Factor specifically agglutinates cells of strain 21 and is a mannan-protein.

No agglutinin could be isolated from strain 21 but an activity was present in cell-free extracts of strain 21 which was specifically adsorbed to strain 5 cells and which inhibited the agglutination activity of the 5-factor. This activity, the 21-factor, was purified 1,200-fold and found to be a glycoprotein which probably contains mannose. The 21-factor, being an inhibitor rather than an agglutinin, is probably univalent. It is homogeneous and has a sedimentation coefficient of 2.9 $S_{20,w}$. It was released from strain 21 cells by trypsin digestion which at the same time destroys the agglutinability of the cells.

Another inhibitor of agglutination was found also in strain 21 cells but this inhibitor was found to be nonspecific in the sense that it was released from all strains (5, 21, 72, 73 and 5 x 21 hybrid) and absorbed to both mating types nonspecifically. The nonspecific inhibitor is heterogeneous, of large molecular weight and is probably also a glycoprotein.

Nonagglutinating mutants of strain 5 were isolated which lacked 5-factor but since they were also asexual and not conditional mutants they could not be studied genetically. In addition, a mutant was isolated which was asexual but still was able to agglutinate. This mutant demonstrates that there are other specific processes besides agglutination involved in conjugation that are not required for growth.

Since the diploid hybrid is nonagglutinative, the diploid was studied to determine if the synthesis of both agglutination factors was repressed or if both were synthesized but were mutually neutralized or inactivated in the cytoplasm. Evidence was obtained which suggested that neither factor was synthesized in the diploid. Neither factor was found alone or present in a 5-factor:21-factor complex in cell-free extracts of the diploid or on the cell wall. Further evidence was obtained for the hypothesis of mutual repression when it was observed that the diploid could synthesize 5-factor when grown into late stationary phase in the presence of yeast extract. Only certain batches of Difco yeast extract were effective. Under these conditions it is possible that the repression mechanism for the synthesis of 5-factor is inactivated. A model was proposed which suggested that each haploid contains a structural gene for its agglutination factor and a regulatory gene for the opposite mating factor. When these genes are present, in apposition, in the diploid, the agglutination factors are mutually repressed.

During this work, Professor T. D. Brock was supported by NSF Grants GB-1964 and GB-6001 and M. A. Crandall was supported by a NIH PHS predoctoral fellowship.

X Department of Biology, McMaster University, Hamilton, Canada. Communicated by Dr. J. J. Miller.

Dr. Alice W. Chen left in March, 1968, for the Department of Developmental Biology, Retina Foundation, Boston, Mass. after a stay of 17 months as a Postdoctorate Fellow here. While with this laboratory she studied the changes in acid proteolytic activity of intact S. cerevisiae cells during sporulation. Proteolytic activity of vegetative cells from presporulation medium was very low, and no change was noted until the cells had been more than 2 hours in potassium acetate sporulation medium, when activity began to increase. Maximum activity was observed after approximately 17 hours in sporulation medium. At this time a large proportion of the cell population was in the tetranucleate stage and spore walls were not yet visible. Activity declined as the spores formed but did not fall to the level characteristic of vegetative cells. Non-sporogenic strains showed no change in activity when placed in sporulation medium. Evidence suggested a peripheral location for the proteinase enzyme involved. A publication describing the work is in press in the Canadian Journal of Microbiology.

Mrs. M. Banerjee is following quantitatively the changes in abundance of the major reserve substances of S. cerevisiae during sporulation and spore germination. Mr. J. L. Seigel is studying the changes in respiratory capacity and the loss of acid-fastness during yeast spore germination. Mr. N. Grewal is comparing the sensitivity of yeast cells and spores to various toxins.

As a long term project, a survey is being made of the sporogenic capacity and loss of spore viability in storage, of yeast strains (mainly industrial) from many sources. To date approximately 250 strains have been tested for ability to sporulate. Cells of these strains removed from sporulation medium are stored under dry conditions and tested periodically for survival with a view to screening out strains having highly-resistant spores. Wide variation in spore survival ability has been found among the strains studied. Some are sufficiently resistant to indicate the feasability of storing certain yeasts in the spore stage for industrial use or in culture collections. Obviously, this would not be practical with polyploids. Although new genetical combinations result from sporulation and conjugation in heterozygous diploids, this would be compensated for to some degree by the availability of a stable source of inoculum. Spore inoculum can be prepared more cheaply and conveniently than lyophilized vegetative cell inoculum. It is of interest that Emeis (European Brewery Conv. Stockholm, 1965, pp. 156-163) has recently concluded that mitotic crossing-over occurs much more frequently than mutation in vegetative yeast. There is consequently no assurance that variation due to recombination of genetical material can be avoided by maintaining diploid cultures in the vegetative state in any event, so perhaps we should give serious consideration to the possibility of putting the well-known ruggedness of the yeast spore to practical use.

Recent publications:

Miller, J. J. Some cultural and staining methods for the observation of meiosis in Saccharomyces. In "Effects of radiation on meiotic systems", published by International Atomic Energy Agency, Vienna, 1968. Pages 177-184.

Miller, J. J. Physiologie de la sporulation chez les levures du genre Saccharomyces. Bull. Soc. Hist. Naturelle, Toulouse, 103:327-339, 1967.

XI Microbiological Institute, Technical University, Zürich, Switzerland. Communicated by Dr. H. K. von Meyenburg.

The following article was recently published in Path. Microbiol. 31: 117-127, 1968 (German with English summary).

"Der Sprossungszyklus von Saccharomyces cerevisiae" by H. K. von Meyenburg.

Summary

The Budding Cycle of Saccharomyces cerevisiae

The sequence of budding and single cell phase during the budding cycle of growing cells of Saccharomyces cerevisiae in dependence on the growth conditions, batch and continuous culture, was determined. The percentage of budding cells and single cells in a population under steady state conditions is a direct value for the duration of the budding phase and single cell phase, respectively. The length of the budding phase is nearly unaffected by the specific growth rate μ (1.5 h at $\mu = 0.4$, 1.85 h at $\mu = 0.085$) under aerobic conditions. The length of the single cell phase on the other hand varies strongly from high values at low growth rates (8.0 h at $\mu = 0.085$) to very small ones at higher μ (0.2 h at $\mu = 0.40$).

The relationship between the physiological state of the populations and the arrangement of the budding cycle under various growth conditions is discussed. A sequence of the formation and degradation of reserve carbohydrates as well as one of the synthesis of the glycolytic and oxidative enzymes is proposed to be closely related to the budding cycle.

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

The following papers related to yeasts have appeared from this laboratory during the past year:

1. Invertase biosynthesis and the yeast cell membrane. J. O. Lampen, N. P. Neumann, S. Gascón and B. S. Montenecourt. 1967. In Organizational Biosynthesis (ed. by H. J. Vogel,

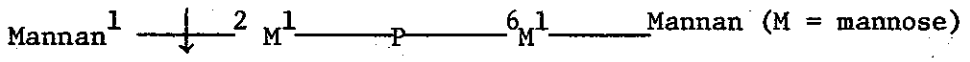
J. O. Lampen and V. Bryson), p. 363. Academic Press, New York.

2. External enzymes of yeast: their nature and formation. J. O. Lampen. Antonie van Leeuwenhoek 34:1-18 (1968).

These two reviews summarize the work of our group on the secretion of invertase by yeasts and yeast protoplasts, the nature of the external and internal enzymes, the action of the new enzyme, phosphomannanase, and finally some ideas on the structure of the yeast wall.

3. Phosphomannanase (PR-factor), an enzyme required for the formation of yeast protoplasts. W. L. McLellan and J. O. Lampen. J. Bacteriol. 95:967 (1968).

This enzyme is necessary for the production of protoplasts from yeast. It can act on the intact cell and releases mannan and mannan proteins (molecular weight greater than 200,000). The enzyme also depolymerizes phosphomannans produced by Hansenula species. It cleaves a mannosidic bond adjacent to a mannose which is also phosphodiester linked through carbon-1. This may be shown schematically as follows:



Evidence is presented that the wall has an outer layer of mannan crosslinked through phosphodiester bonds.

4. Purification of the internal invertase of yeast. S. Gascón, J. O. Lampen. J. Biol. Chem. 243:1567 (1968).

5. Comparative study of the properties of the purified internal and external invertases from yeast. S. Gascón, N. P. Neumann and J. O. Lampen. J. Biol. Chem. 243:1573 (1968).

The internal invertase of a strain of yeast has been isolated in a homogenous state. Its molecular weight is 135,000, the same as that of the protein moiety of the external enzyme, and its specific activity per mg of protein is the same as that of the external enzyme. However, their amino acid compositions are strikingly different. The internal enzyme contains no carbohydrate; the external one 50% mannan and 3% glucosamine. Their enzymatic and kinetic characteristics are essentially identical. The enzymes are immunologically related, and sucrose-negative mutants lack both external and internal invertase activity.

If the differences in amino acid composition of the two proteins are taken to indicate that the enzymes are aggregates of different subunits, at least one of which is identical in the internal and external forms, the close similarity of all of the other properties would then suggest a precursor-product relationship for the two enzymes.

XIII Departments of Biochemistry and Electron Microscopy, Royal Dental College, Århus, Denmark, and Department of Biophysics, Statens Seruminstitut, Copenhagen, Denmark. Communicated by Dr. Sven Darling.

The following is an abstract of a report to be given by Dr. J. Theilade at the annual meeting of the Scandinavian Society for Electron Microscopy

in Stockholm in early June of 1968.

Kinetic and morphologic observations on Saccharomyces cerevisiae during spheroplast formation. SVEN DARLING, JØRGEN THEILADE and AKSEL BIRCH-ANDERSEN.

A strain of S. cerevisiae was studied, the cells of which are elongated under the culture conditions used. By digestion of cell walls with snail enzyme the cells form spheroplasts through a transient state we term prospheroplast. The prospheroplast is able to lyse as does the spheroplast, but will retain the shape of the intact cell if osmotically protected. Cultures of S. cerevisiae Hansen no. 983 were grown in liquid medium and harvested after 18 hours. After pretreatment of the cells with β-mercaptoethanol, prospheroplasts and spheroplasts were prepared by digestion with snail enzyme in tris buffer (pH 7.5) containing 0.6 M KCl. Samples were prepared for sectioning and electron microscopy at regular intervals up to the time, at which all cells were converted to spheroplasts. Structures resembling cell wall material were prepared from prospheroplasts and spheroplasts using a modification of the method described by Ottolenghi (Comp. Rend. Trav. Lab. Carlsberg 35:363, 1966). These specimens were shadow cast for electron microscopy. After a certain period all cells were transformed to prospheroplasts whereas the spheroplast formation started later depending on the enzyme concentration. In sections the prospheroplasts appeared to be formed by detachment of the cell walls. Both the prospheroplasts and the spheroplasts showed asymmetric cytoplasmic membranes in which the outer leaflets appeared coated with a dense hairy layer. The shadowed material revealed structures resembling bud scars suggesting that they belonged to the cell walls. The experiments suggest that the cytoplasmic membrane after the enzyme digestion retains a coating which is rigid in the prospheroplast but loses rigidity when the cell is transformed to a spheroplast.

XIV Hopkins Marine Station, Pacific Grove, California. Communicated by Dr. Norval A. Sinclair.

The following is a brief summary of research in progress.

During the course of investigations on glucose metabolism in the obligate psychrophile Candida gelida the following observations have been made. Resting cells from young glucose-grown shake cultures do not ferment glucose whereas resting cells from older cultures ferment this sugar readily. Young cultures however can be induced to ferment glucose if placed under anaerobic conditions. These observations suggest that fermentation of glucose by C. gelida is an inducible process regulated at least in part by availability of oxygen. When oxygen becomes limiting in glucose grown shake cultures and if sufficient glucose remains, the cells adapt to a fermentative type of metabolism. This is further substantiated by observations that resting cells from glucose-limited cultures do not ferment glucose irrespective of the age of the culture.

It has also been observed that induction of fermentation occurs only within the temperature limits of growth of the organism. Induced cells ferment glucose optimally at 25C although growth does not occur at this temperature. Induction does occur at 12C and 20C. The latter temperature

Heikki Suomalainen and A. J. A. Keränen. Valine, leucine and isoleucine as precursors of branched fatty acids in yeast. Suomen Kemistilehti 40B, 288-289 (1967).

When valine, leucine and isoleucine are added to a fermentation medium, the yeast produces, in addition to isobutyl, isoamyl and opt. act. amyl alcohols, isobutyric, isovaleric and α-methylbutyric acids. The formation of these branched-chain fatty acids seems to indicate that not only the yeast, but also the raw material influences the formation of the aroma components of fermented beverages. On the other hand, these amino acids do not have any influence on the formation of esters.

Heikki Suomalainen, Timo Nurminen and Erkki Oura. Isolation of the plasma membrane of yeast. Suomen Kemistilehti 40B, 323-326 (1967).

Yeast plasma membrane preparations were obtained by centrifugation, in the presence of magnesium, of the residue remaining after controlled enzymatic digestion of a carefully isolated cell wall fraction of baker's yeast. The chemical composition of the original cell wall fraction was analyzed, the changes in the chemical composition and the activities of ATPase, acid phosphatase and saccharase, located on the surface of the cell, were followed during the enzymatic digestion. The progress of the digestion was investigated also by phase-contrast and electron microscopy which revealed the presence of very thin membrane-like bodies in the digested preparation. Preliminary studies suggest a lipoprotein nature of the plasma membrane. The lipid components were examined more in detail.

XVI Lehrkanzel für Biochemische Technologie, Institut für Lebensmitteltechnologie, Hochschule für Bodenkultur, Wien, Austria. Communicated by Dr. H. Klaushofer.

Preparation of a monospecific anti-Saccharomyces carlsbergensis serum for biological quality control. By K. Dorfwirth and H. Klaushofer.

The use of serological procedures requires the employment of monospecific antisera not only in taxonomy but also in biological quality control. The serological behavior of the yeast Sacch. carlsbergensis, which is normally used as culture yeast in continental breweries, is related on the basis of its antigenic make-up even to those yeasts, which evoke infections during the brewing process.

Earlier works have shown that these antigenic relationships are the reason that a quick fluorescent detection of "wild yeasts" in brewery yeast is very difficult. This paper deals with experiments to obtain a monospecific anti-Sacch. carlsbergensis serum in sufficient yield by TSUCHIYA's method.

For this work it was first necessary to choose a representative strain out of a number of industrial strains by the methods of numerical taxonomy, with respect to point 3 of the resolution of the I. International Symposion on Yeasts (Smolenice 1964).

The special procedures for group specificity, proposed principally by KOCKOVA-KRATOCHVILOVA, were applied. With this representative strain

is the apparent maximal growth temperature of C. gelida. At 20C however the rate of induction is about one-half the rate at 12C.

Induction of fermentation in resting cell suspensions is inhibited by DL-ρ-fluorophenylalanine and actidione, both of which are inhibitors of protein synthesis.

XV Research Laboratories of the State Alcohol Monopoly (Alko), Helsinki, Finland. Communicated by Dr. Heikki Suomalainen.

Heikki Suomalainen and A. J. A. Keränen. Keto acids formed by baker's yeast. J. Inst. Brewing 73, 477-484 (1967).

The keto-acids formed in baker's yeast during anaerobic fermentation and aerobic growth were identified, by means of thin-layer chromatography, as their 2,4-dinitrophenylhydrazones and as corresponding amino acids after reduction of the hydrazones. Pyruvic acid, α-ketoglutaric acid and p-hydroxyphenylpyruvic acid, were found in abundance. In addition, the following keto acids were present in estimated descending order of magnitude: α-keto-β-methylvaleric acid, α-keto-isovaleric acid, α-keto-isocaproic acid and β-phenylpyruvic acid, and small amounts of α-ketobutyric acid, oxalacetic acid and α-keto-γ-methiolbutyric acid. Under anaerobic conditions a somewhat larger amount of keto acids accumulated in the medium than under aerobic conditions, but in both cases the amounts rose markedly as the content of seed yeast increased.

Pentti Ronkainen, Vilho Arkima and Heikki Suomalainen. The identification of carbonyl compounds in beer. J. Inst. Brewing 73, 567-570 (1967).

Carbonyl compounds were separated from bottom-fermented lager beer by extraction with a diethylether-pentane mixture and precipitation from the extract with 2,4-dinitrophenylhydrazine. The diketones, diacetyl and 2,3-pentanedione, were identified as bishydrazones by thin-layer chromatography with benzene-petroleum ether-ethyl acetate (34:5:1) as solvent and Silica Gel HF_{254} as adsorbent. For the identification of the aldehydes, the hydrazone precipitate was treated with ozone in formic acid solution and the aldehydes were determined as their corresponding carboxylic acids by iso-thermal gas chromatography on a NEGS column. Aldehydes were found in the following order of magnitude: acetaldehyde, isobutyraldehyde, isovaler- and/or opt. act. valeraldehyde (2-methylbutyraldehyde), valeraldehyde and butyraldehyde.

Heikki Suomalainen, Virve Christiansen and Erkki Oura. The increasing effect of mannose used as carbon source on the saccharase activity of baker's yeast. Suomen Kemistilehti 40B, 286-287 (1967).

The saccharase activity as well as the mannan content of baker's yeast grown with mannose as carbon source are clearly higher than when the yeast is grown with glucose as substrate. The saccharase activity of the yeast grown with mannose is nearly three times that of the yeast grown with glucose.

rabbits were immunized and later on the globulin fraction separated from the serum. Sephadex G-200 column chromatography was used for further subfractionation to obtain two main fractions. Antibody activity of each fraction was examined by agglutination and precipitation tests with the yeasts related to Sacch. carlsbergensis.

After determination of the useful fractions the antiserum was stepwise adsorbed. This antiserum, reacting only with the homologous strain, was finally labeled with fluoresceinisothiocyanate.

A discussion of this work was presented at the IId International Symposium on Fermentation, held in Leipzig (DDR) from May 27-June 1, 1968.

XVII Department of Agriculture, University of Mie, Kamihama-Cho, Tsu-City, Mie Prefecture, Japan. Communicated by Dr. Morio Akaki.

The following paper has been published:

Studies on the Brewing of Sake using Pure Cultures of Saccharomyces sake instead of "Moto". (V) Prosperity and Decay of Yeasts during the Moromi-Making Process; R. Miyazaki, O. Nagano, H. Yoshida, and M. Akaki. Jour. Soc. Brewing, Japan, 62, 168-172 (1967).

SUMMARY

Sake was brewed by using a pure culture of sake yeast instead of Moto. The pure culture of yeast was prepared by cultivating sake yeast Kyokai No. 7 in a molasses medium containing malic acid, urea and salts in a propagation tank described in the preceding paper. In this sake brewing process one- and two-day "Odori" periods were provided for moromi making. Sake was also brewed by the ordinary moto process using "Sokujomoto", as a control. In this ordinary process a one day "Odori" period was provided for moromi making. Comparative studies between the ordinary moto process and the pure cultured yeast process were carried out by measuring the number of yeast cells in moromi mash, the amounts of the components in moromi mash, Baume and pH of moromi mash, and the appearance of moromi mash during the moromi making process. Results obtained were as follows:

1. Changes in the number of yeast cells in each of the moromi mashes were closely related to the temperature of moromi.

Yeast cell numbers in each of the moromi mashes tended to decrease gradually in the same manner after they reached their maximum, although some differences were observed at the initial stage of moromi making process. The maximum yeast cell concentrations in each of the moromi mashes were found in a range of 16.3-17.1 X 10^7/ml.

2. The yeasts in each sample, which was taken periodically from the moromi mashes during moromi processing, were classified according to the TTC-agar overlay method. The ratio of cell numbers of sake yeast Kyokai No. 7 to the whole cell numbers of yeast in each moromi mash sample was investigated.

In general, this ratio was higher in the moromi mashes made with pure culture of sake yeast than in the ordinary moromi mash.

3. The degree of increase in yeast cell numbers of moromi mash was far greater in the normal mashes made with pure culture of sake yeast than in the ordinary moromi mash.

4. No large differences were found in the amounts of components (such as amino acids and reducing sugars, and in the pH of moromi mash) between the moromi mashes made with a pure culture of sake yeast and the ordinary moromi mash, but the Baume degree and alcohol content in the former moromi mashes tended to be somewhat greater than in the latter.

5. As for the appearance of moromi mash, no large difference was found between the moromi mash, but some difference was found between the two types of moromi mashes at the first stage of the moromi process.

XVIII The Australian Wine Research Institute, Private Mail Bag No. 1, Glen Osmond, South Australia, 5064. Communicated by Dr. B. C. Rankine.

The examination of Saccharomyces for their use in wine fermentations is being continued and the formation of higher alcohols has been examined by gas chromatography. Yeasts differ considerably in the amounts of n-propanol, iso-butanol and iso- and active- amyl alcohol which they produce, and the range of amounts of these compounds found in wine can be attributed, at least in part, to the yeast strain alone. The differences between yeasts appear to be unrelated to taxonomy, and other genera, such as Saccharomycodes, Kloeckera, Hansenula and Schizosaccharomyces, also produced higher alcohols, even when some of them grew as films without visible fermentation. The results of this study have now been published - "Formation of higher alcohols by wine yeasts and relationship to taste thresholds", B. C. Rankine, Journal of the Science of Food and Agriculture (1967) 18:583-589.

XIX Technical University of Agriculture, Dept. of Viticulture, Lednice, Czechoslovakia. Communicated by Dr. E. Minárik.

V. Švejcar: Contribution to the classification of the yeast flora of vineyards of the School Farm of the Technical University of Agriculture in Lednice (Moravia). 84 pages incl. 14 tables, 3 graphs and 14 photographs. Dissertation, Technical University of Agriculture, Brno 1967.

The ecology of natural wine yeast species in the locality of Lednice in South Moravia was studied. The yeast association was examined during 3 years in soils of yielding and not yielding vineyards, on various organs of the vine, in grape juice and young wine.

It could be repeatedly confirmed that sporogenous yeast, first of all Saccharomyces cerevisiae var. ellipsoideus are found not only on secondary habitats in the wineries, but, above all, from primary habitats, e.g. from soil and grapes.

Compared with other vine regions in Czechoslovakia an important number of asporogenous yeasts, viz. Candida mycoderma and Candida zeylanoides, were found. These film-forming yeasts represent a potential

danger for young wines causing "wine flower".

It was confirmed that the occurrence of dominant species of yeasts and yeast-like microorganisms is influenced by climatic conditions, above all by rainfall and average temperature during the vegetation period.

XX Brief News Items

1. Laboratory of Microbiology, Gulbenkian Institute of Science, Oeiras, Portugal. Communicated by Dr. N. van Uden.

   a. Miss Manuela Vidal Leiria has returned from the University of California in Davis where she obtained a M.A. degree in Microbiology. Her thesis, prepared under the guidance of Professor H. J. Phaff, was summarized in an earlier News Letter (XVI, 25-26, 1968). She is now working on inositol metabolism in yeasts.

   Dr. Terence G. Watson, formerly at the Department of Biochemistry of the University of Birmingham, joined our group on a post-doctoral fellowship to work on maintenance and yield analysis in the chemostat.

   b. Publications in the press

   H. R. Buckley & N. van Uden, Five new Candida species, Mycopathologia et Mycologia Applicata.

   N. van Uden & S. Windisch, Candida friedrichii sp. n. a melibiose fermenting yeast, Antonie van Leeuwenhoek.

   N. van Uden, P. Abranches & C. Cabeça-Silva, Temperature Functions of thermal death in yeasts and their relation to the maximum temperature for growth, Archiv für Mikrobiologie.

   N. van Uden, Yield and maintenance analysis in the chemostat: a tool for metabolic studies of growing cells, Archiv für Mikrobiologie.

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

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

   1. Hwang, Y. L., Bhattacharjee, J. K. and Lindegren, C. C. A localized crossover between the centromere and the MZ_1 locus in Saccharomyces. Canadian Journal of Genetics and Cytology 9: No. 2, 279-286 (1967).

   2. Shult, Ernest E., Lindegren, G. and Lindegren, C. C. Hybrid specific linkage relations in Saccharomyces. Canadian Journal of Genetics and Cytology 9: No. 4, 723-759 (1967).

3. Lindegren, Gertrude, Courtis, William S. and Shult, Ernest E. Linked genes arising among multiple mutants in EMS-treated Saccharomyces. Canadian Journal of Genetics and Cytology. Accepted for publication.

3. Single-Cell Protein. Edited by Richard I. Mateles and Steven R. Tannenbaum. The MIT Press of the Massachusetts Institute of Technology.

The use of single-cell protein (SCP) derived from unicellular organisms such as bacteria, yeasts, algae, and fungi is a promising answer to the problem of developing low-cost protein sources for the alleviation of protein malnutrition. The importance of such an innovation, were it to come about, is obvious. In order to discuss and evaluate the prospective role of SCP in world food supply, a number of authorities gathered at M.I.T. for a three-day conference to consider the nutritional, technological, economic, sociological, and political problems to be overcome in developing SCP into a food source of significant impact.

Based upon the papers presented at the conference, this volume contains contributions from distinguished authorities in many interested fields. It brings together in one place for the first time a great deal of research pertinent to the present and projected use of SCP as a food or food supplement, treating four major aspects of the problem: the need for such a food material, biochemical and nutritional considerations, possible method of production, and the social, political, and psychological impact the development of such a food source would have.

The editors of the collection are members of the Department of Nutrition and Food Science at the Massachusetts Institute of Technology.

Contributors:
Aaron M. Altschul, Antonio Bacigalupo, Zeki Berk, T. L. V. Blair, Ricardo Bressani, Lester Brown, H. J. Bunker, John R. DeZeeuw, G. H. Evans, Arthur E. Humphrey, Pen-chieh Ko, Paul Lachance, John Litchfield, George C. Lodge, Allen G. Marr, Richard I. Mateles, John McKenzie, Sanford A. Miller, Hamish N. Munro, Bernard L. Oser, W. J. Oswald, Henry Peppler, Nevin S. Scrimshaw, Steven R. Ta-nenbaum, Daniel I. C. Wang, Koichi Yamada.

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4. Yeast Genetics Conference 1968.

In connection with the XII International Genetics Congress to be held in Tokyo, this conference is planned to meet in Osaka from 2nd to 5th of September, 1968. The conference will comprise the yeast genetics in a broadest sense. So, any one interested in this field is invited to come and participate in the talks. Osaka is located about 560 kilometers (=360 miles) to the west of Tokyo, and it takes a 3-hour ride by the super express train of the National Railroad System or a 1.5-hour flight by inland airlines. Person in charge of further information is: Dr. Susumu Nagai, Biological Laboratories, National Women's

University, Kitauoya Nishimachi, Nara, Japan.

5. Drs. M. W. Miller and H. J. Phaff will be collecting yeasts from natural habitats along the west coast of the North American Continent, starting in Anchorage, Alaska, and during the trip back to Davis, California. Cooperating in the project are Dr. M. Yoneyama from Hiroshima University and Mr. M. Soneda, from the Nagao Institute in Tokyo. The project is sponsored by the National Science Foundation and by the Japanese Society for the Promotion of Science. The collection will take place from June 10-August 1, 1968.

6. Dr. N. J. W. Kreger-van Rij (Bacteriology Laboratory, University of Groningen, Oostersingel 59, Groningen, Holland) writes: The following article will appear in the May issue of Mycologia: "Shape and structure of the ascospores of Hanseniaspora uvarum" by N. J. W. Kreger-van Rij and Donald G. Ahearn.