Y E A S T

A News Letter for Persons Interested in Yeast

Official Publication of the
International Commission on Yeasts and Yeast-like Microorganisms
of the International Association of Microbiological Societies (IAMS)

June 1973 | Volume XXII, Number 1

Editor
Herman J. Phaff, University of California, Davis, California 95616

Associate Editors

Anna Kocková-Kratochvilová
Slovak Academy of Sciences
Bratislava, Czechoslovakia

Susumu Nagai; National Women's University; Kita-Uoya-Nishimachi ~~Joto-Ku Furuichi Kitadori 4-1; Shiei-jutaku 1-3 Osaka 536,~~ NARA 630, Japan

Richard Snow
Dept. of Genetics, Univ. of California
Davis, Calif. 95616

Torsten O. Wikén
Lab. for Microbiology
Techn. University, Delft, Holland

****

	Page
D. Yarrow, Delft, The Netherlands	1
C. P. Kurtzman, Peoria, Illinois	1
J. W. Fell, Miami, Florida	2
A. Kocková-Kratochvílová, Bratislava, CSSR	2
V. K. Jain, Frankfurt/Main, Germany	4
B. A. Siddiqi, Blindern, Norway	5
Norman E. Gentner, Ontario, Canada	6
Takashi Ito, Tokyo, Japan	8
Sayaka Nakai, Chiba, Japan	9
A. M. A. ten Berge, Utrecht, The Netherlands	10
Norman R. Eaton, Brooklyn, New York	11
F. K. Zimmermann, Darmstadt, Germany	12
Eckhart Schweizer, Wurzburg, Germany	13
Reed Wickner, Bronx, New York	14
H. de Robichon-Szulmajster, Gif-Sur-Yvette, France	15
H. Heslot, Paris, France	16
M. W. Miller, Davis, California	16
Harlyn O. Halvorson, Waltham, Massachusetts	17
A. Sommer, Davis, California	17
H. J. Phaff, Davis, California	18
J. M. Bastide, Montpellier, France	20
J. R. Villanueva, Salamanca, Spain	20
K. Beran, Prague, CSSR	21
J. O. Lampen, New Brunswick, New Jersey	22
N. P. Elinov, Leningrad, USSR	24
Byron F. Johnson, Ottawa, Canada	25
E. Azoulay, Marseille, France	26
Edward Spoerl, Charlottesville, Virginia	26
H. Suomalainen, Helsinki, Finland	27
S. P. Meyers, Baton Rouge, Louisiana	28
V. Kostov, Sofia, Bulgaria	28
J. T. Rice, Milwaukee, Wisconsin	29
A. Fiechter, Zurich, Switzerland	29
Yataro Nunokawa, Tokyo, Japan	30
E. Minárik, Bratislava, CSSR	30
D. M. Levine, Cambridge, Massachusetts	31
R. V. Chudyk, Ontario, Canada	31
Brief News Items	33

I. Centraal Bureau voor Schimmelcultures, Yeast Division, Julianalaan 67A, Delft, The Netherlands. Communicated by D. Yarrow.

The following new species have been deposited in the CBS collection since the last issue of the Yeast News Letter.

Candida iberica CBS 6391 (type), CBS 6408, CBS 6409, CBS 6410, CBS 6411
C. Ramirez and G. Gonzáles, Can. J. Microbiol. 18:1778 (1972).

Candida subtropicalis CBS 6465 (type)
T. Nakase, Y. Fukazawa and T. Tsuchiya, J. gen. Appl. Microbiol. 18:349 (1972).

Debaryomyces formicarius CBS 6454 (type), CBS 6455
V. I. Golubev and I. P. Babjeva, J. gen. Appl. Microbiol. 18:249 (1972).

Kazachstania viticola CBS 6463
R. D. Zubkova, Botanical Magazine of the Herbarium of the Institute of Botany, Academy of Science Kazachstan SSR 7:53 (1971) (this strain appears to be identical with Saccharomyces dairensis Naganishi according to our observations).

Saccharomycopsis crataegensis CBS 6447 (Holotype), CBS 6448 (isotype)
C. P. Kurtzman and L. J. Wickerham, A.v. Leeuwenhoek 39:81 (1973).

Schizosaccharomyces slooffiae CBS 6207, CBS 6233
M. S. Kumbhojkar, Curr. Sci. 41:151 (1972). (Both strains were indicated as the type in the description of this species. CBS 6233 did not sporulate on receipt by the CBS but CBS 6207 did. Therefore CBS 6207 is a better representative of this species than CBS 6233.)

II. United States Department of Agriculture, Northern Regional Research Laboratory, 1815 North University Street, Peoria, Illinois 61604, U.S.A. Communicated by C. P. Kurtzman.

The following are abstracts of two papers. Both will be submitted for publication at this time.

Kurtzman, C. P., R. F. Vesonder and M. J. Smiley. Formation of extracellular 3-D-hydroxypalmitic acid by Saccharomycopsis malanga comb. nov.

Summary: All strains of S. malanga were isolated from Oriental fermented food starters and included one strain previously described as Hansenula malanga. Extracellular 3-D-hydroxypalmitic acid was formed by all cultures and this appears to be the only known yeast to produce this fatty acid in the free form. The compound is inhibitory to Vibrio tyrogenus but not to several other microorganisms tested. A key to the six currently recognized species of Saccharomycopsis is given.

Kurtzman, C. P., R. F. Vesonder and M. J. Smiley. Formation of extracellular C_{14}-C_{18} 2-D-hydroxy fatty acids by species of Saccharomycopsis.

Summary: Eighteen of 19 strains of Saccharomycopsis fibuligera and one of two strains of S. capsularis produced mixtures of C_{14}-C_{18} 2-D-hydroxy acids. Generally, the C_{16} acid was most abundant followed by the C_{18} homolog. The only other yeast known to produce these acids free in the culture medium is Hansenula sydowiorum. One of the S. fibuligera cultures examined was recently described as Candida lactosa.

The following recently published papers may also be of interest to readers:

Kurtzman, C. P., M. J. Smiley and F. L. Baker. 1972. Scanning electron microscopy of ascospores of Schwanniomyces. J. Bacteriol. 112:1380-1382.

Abstract: Ascospores of the four recognized species of Schwanniomyces were examined by scanning electron microscopy. Spores of S. alluvius, S.

castellii, and S. occidentalis, which were essentially identical, had abundant, long protuberances and wide, thin equatorial rings. The two known strains of S. persoonii differed from the other species as well as from each other. One strain had spores with a wide ring but only a few short protuberances; spores from the second strain were covered with crater-like depressions and had a narrow ring. Also examined were spores of Schwanniomyces hominis (= Saccharomyces rosei) which lacked a ring and were covered with short irregularly shaped protuberances, a finding consistent with the morphology of spores from other strains of S. rosei.

Kurtzman, C. P., J. C. Gentles, E. G. V. Evans, M. E. Slodki and R. M. Ward. 1973. Growth of Hansenula holstii on Cadavers. Appl. Microbiol. 25:184-186.

Abstract: Growth of a yeast was observed on prosected cadavers used for demonstration purposes in a medical school. An asporogenous yeast was isolated and identified as an atypical form of Hansenula holstii by analysis of the extracellular polysaccharide. The isolate showed resistance to embalming fluid but was eventually eradicated by addition of picloxidine digluconate to the fluid.

III. Institute of Marine Science, University of Miami, Rickenbacker Causeway 1, Miami, Florida 33149, U.S.A. Communicated by J. W. Fell.

The following paper will appear in a forthcoming issue of the Canadian Journal of Microbiology.

Fell, J. W., I. L. Hunter and A. S. Tallman. 1973. Marine basidiomycetous yeasts (Rhodosporidium spp.n) with tetrapolar and multiple allelic bipolar mating systems.

Abstract: Studies of the mating systems of basidiomycetous yeasts from oceanic regions demonstrate that Rhodosporidium bisporidiis sp.n. and Rhodosporidium dacryoidum sp.n. have tetrapolar mating factors. Rhodosporidium infirmo-miniatum sp.n. is bipolar with three known alleles. The formal description of these three species is presented, as well as that of Rhodosporidium capitatum sp.n., a self-sporulating form. Rhodosp. capitatum and Rhodosp. bisporidiis are distinguished by the formation of basidia with terminal sporidia that develop on primary sporidia. The sporidia are not forcefully ejected. Rhodosp. infirmo-miniatum has short basidia with terminal sporidia, but lacks primary sporidia. Rhodosp. dacryoidum has 2-4 celled basidia with lateral sporidia. Rhodosp. capitatum, Rhodosp. infirmo-miniatum and Rhodosp. bisporidiis produce endospores. The taxonomic status of these species, and of the basidiomycetous yeast-like genera, is discussed.

IV. Institute of Chemistry of the Slovak Academy of Sciences, Laboratory of Microbiology, Bratislava, Dúbravská cesta, ČSSR. Communicated by A. Kocková-Kratochvílová.

The following articles will soon be published:

1) Kocková-Kratochvílová, A. and A. K. Wegener. The relationships within the genus Rhodotorula Harrison. Zentralblatt f. Bakteriologie. II. Abt. (in press, in German).

A collection of 99 Rhodotorula strains was studied by means of 60 tests, which included morphological, physiological, biochemical and serological properties. The experimental results were subjected to computer analysis using matching coefficients and taxonomic distances. Cluster analysis was carried out using average linkage method. Twelve phenons were formed, which were grouped into three clusters and four individual

border-line strains. These groups were named after the most frequently participating species: Minuta, Rubra and Glutinis. Many colour mutants, prepared by UV irradiation, were included and their taxonomic position was discussed. The genus Rhodotorula seemed to be homogeneous, formed clusters, and showed the taxonomic continuum. Comparison of the results with those obtained with respect to their GC content revealed good agreement:

Cluster	GC mol % in DNA
Minuta	50 - 55
Rubra	55 - 60
Glutinis	60 - 67

The taxonomic position of the species Rh. aurantiaca was discussed.

2) Kocková-Kratochvílová, A. The future trend in yeast diagnostics. Uspechi mikrobiologiji 9 (in press, in Russian).

The tedious process of recent yeast diagnostics leads to the searching for quick tests and objective methods in the field of yeast taxonomy. Numerical taxonomy seems to be one of the most objective methods in classification. Individual steps in the process of numerical taxonomy, the computation of similarities, cluster analysis and dendrogram construction were discussed. Two examples were introduced: the heterogeneous genus Torulopsis and the homogeneous genus Rhodotorula. The possibility of realizing the full automation of yeast diagnostics in future was considered.

3) Kocková-Kratochvílová, A. and M. V. Blagodatskaja. The problem of the ploidy in numerical taxonomy. Biológia (Bratislava) (in press, in English).

The phenotypes of ten ploidal strains of the species Saccharomyces uvarum were evaluated: two haploids (a and α), three diploids (aa, αα, aα), two triploids (aαα, aaα), two tetraploids (aaαα, αααα), and one hexaploid (aaαααα). The genome of these strains was re-evaluated by a morphological method, exploiting the fact, that the cell volume was proportional with the increasing ploidy. The logarithmic transformation of cell sizes showed a linear correlation. The above-mentioned ten strains were classified numerically together with 23 strains of S. uvarum and with 26 productive strains, used in middle European breweries. Haploids and diploids showed greatest similarity to S. uvarum group, triploids and tetraploids to productive brewing yeasts. It can be concluded, that the increasing ploidy can show a way, how new productive strains can be searched for.

4) Kocková-Kratochvílová, A. The comparative taxonomy of the genus Torulopsis Berlese (prepared for publication).

A set of 121 strains of yeasts, originally described as Torulopsis, was studied by means of 63 morphological, physiological and serological characters and was subjected to numerical analysis. Matching coefficient of Sokal and Michener and the average linkage cluster analysis served for the grouping of strains into nine phenons, formed on the 80% similarity level: 1. Stellata; 2. Datilla; 3. Colliculosa; 4. Molischiana; 5. Pinus-Schatavii-Glabrata; 6. Versatilis; 7. Gropengiesseri; 8. Wickerhamii-Kruisii-Candida; 9. Holmii. Resulting data were compared with published results of GC content in DNA.

New species was described:

1) Kocková-Kratochvílová, A. and D. Ondrušová. Torulopsisarten aus den Oberflächen höherer Pilze. Torulopsis kruisii n.sp. und Torulopsis schatavii n.sp. Biológia (Bratislava) 26:477-485, 1971.

2) Blagodatskaja, V. M. and A. Kocková-Kratochvílová. The heterogeneity of the species Candida lipolytica. Candida pseudolipolytica n.sp. and C.

lipolytica var. thermophila n.var. Biológia (Bratislava) 27 (in press, in English).

56 strains of Candida lipolytica, isolated from various samples of oil soaked soils in USSR were compared numerically. Four phenons were built on the 90% similarity level. One of them was described as a few species and another as a new variety.

3) Kocková-Kratochvílová, A., H. Weide and M. Havelková. Prototheca hydrocarbonea n.sp. Life cycle, biochemical and electronoptical study (prepared for publication).

Four strains of Prototheca, a colourless Alga, were isolated by Heyer from oil slime in GDR. Two of them were identified as a new species Prototheca hydrocarbonea. Electronoptical studies of the life cycle were recorded using ultra-thin sections, freeze-etching and scanning techniques.

V. Institut für Biologie, Abteilung für Biophysikalische Strahlenforschung, Paul-Ehrlich-Str. 15 u. 20, 6000 Frankfurt/Main, West Germany. Communicated by V. K. Jain.

The following papers summarize the work being done on Yeast in this Department.

In Press:

Influence of Energy Metabolism on the Repair of X-ray Damage in Living Cells. III. Effect of 2-Desoxy-D-Glucose on the Liquid-Holding Reactivation in Yeast.
V. K. Jain, W. Pohlit and S. C. Purohit.
(Accepted for publication in Biophysik, 1973)

SUMMARY

Effects of the glucose antimetabolite 2-deoxy-D-glucose (2DG) on liquid-holding reactivation (LHR) in X-irradiated yeast were studied. It has been observed that LHR in respiratory-deficient mutants is completely inhibited at a molar concentration ratio 2DG: glucose equal to 1, whereas for the wild type this ratio is 10. Significance of these results for the radiochemotherapy of tumours is discussed.

Published:

Influence of Energy Metabolism on the Repair of X-ray Damage in Living Cells. II. Split-dose Recovery, Liquid-holding Reactivation and Division Delay Reversal in Stationary Populations of Yeast.
V. K. Jain and W. Pohlit.
Biophysik 9:155-165, 1973

SUMMARY

Split-dose recovery (SDR), liquid-holding reactivation (LHR) and division delay reversal (DDR) in X-irradiated stationary cell populations of Saccharomyces cerevisiae were investigated in buffer and in the presence of glucose.

In buffer, all the three recovery phenomena show sigmoid type of kinetics but with some quantitative differences. DDR is completed in relatively shorter time as compared to LHR or SDR. The time needed for the completion of LHR and SDR is about the same but LHR shows a longer lag time. LHR and DDR depend upon temperature in a similar way.

On adding glucose to the medium, an increase in the rate of LHR, a delay in the onset of SDR and a transient increase in the radiation sensitivity after the first irradiation are observed.

VI. Institute of General Genetics, University of Oslo, Post Office Box 1031, Blindern, Norway. Communicated by B. A. Siddiqi.

Radiation Studies with Monosomics and Disomics of Yeast.
B. A. Siddiqi

Since cells with unbalanced genomes more often turn into malignant growth than cells with normal compliments, the radiation effect on such cells will be of interest. We therefore, investigated two types of yeast strains, monosomics (2n-1) and disomics (n+1).

Altogether 14 strains of Saccharomyces cerevisiae were utilized. The genotype of the diploid strain (X951) and the five monosomic strains (X951-13, X951-20, X951-22, X951-23 and X951-25) have been described by Strømnaes (1968) and by Øyen (1973). The disomic strains (Y242, Y243, and Y246) are the same as those used by Goldberg et al. (1972). The standard wild-type haploid S288C and wild-types haploids X2180-1B (α mating type) and X2180-1A (a mating type), as well as the wild-type diploid obtained by crossing X2180-1B and X2180-1A, were employed for comparative study of radiation sensitivity. The haploid strain X1687-16C has been described by Strømnaes and Mortimer (1968).

The procedure for determining the survival curves is the same as described by Resnick (1969). Cells were irradiated with X-ray from a beryllium window tube (Machlett OEG 60) at dose rates of 9.3 KR/min or 42 KR/min. The ultraviolet source was a Hanovia model 12 low pressure mercury tube giving 80% of its light at 2540 Å. For testing sensitivity, the chosen dose rate was 11 erg/mm^2/sec.

At low dose rate of X-ray and for the dosages employed, all five monosomics showed no relative loss of viability in comparison with the parental strain X951 or the wild-type diploid strain. All the monosomics and diploid strains have curves with long shoulders. The three disomics behaved differently from one another. The response of strain Y246 was similar to that of a diploid strain with a long shoulder while strain Y242 was more sensitive than strain Y246 but was less sensitive than Y243 which in turn was more sensitive than the wild-type haploid.

At higher dosages the X-ray response of the monosomics were again very similar to that of the parental strain X951 or the wild-type diploid strain. The survival curves of all the monosomics had typically long shoulders like the diploid strains. Monosomic X951-22 was the most sensitive among all the monosomics studied. The loss of whole or a part of the large chromosome VI from the genome or some other factor like defective repair mechanism could be responsible for this sensitivity.

All three disomics behaved differently from one another. Strain Y246 gave a sigmoidal survival curve while the survival curve of strain Y242 was very similar to that of wild-type haploid. Strain Y243 was very sensitive towards X-rays.

With UV irradiation the following picture emerged. When five monosomic strains were irradiated, the survival curves showed great similarity to that of the parental diploid strain X951. All the five monosomics gave survival curves with long shoulders. The results obtained with three disomic strains demonstrated that the disomic Y246 was the least sensitive as compared with the other two disomics. Strain Y243 was more sensitive than the standard wild-type haploid (X288C) and another haploid strain (X1687-16C). Strain Y242 was less sensitive than the wild-type haploid.

On the basis of their X-ray and UV sensitivities, one could conclude that monosomics by the loss of one chromosome do not alter their capability of repair from X-ray or UV damage. By comparing the radiation sensitivity of disomics towards X-ray and UV, one can see that strain Y246 was resistant to both X-ray and UV, strain Y242 was resistant to UV but sensitive to X-rays, strain Y243 seemed to be sensitive to both X-rays and UV. The most interesting strain is Y242 which is sensitive to X-rays but not sensitive to UV. The conclusions

regarding the disomic strains are that strain Y243 is defective in a common pathway of X-ray and UV repair mechanisms, while strain Y246 has efficient repair mechanisms for both X-ray and UV damage. Strain Y242 has X-ray repair mechanism equal to the standard wild-type haploid, but a more efficient UV repair mechanism.

This conclusion supports the hypothesis that in yeast there are mutants that are (i) sensitive to UV but resistant to X-rays, (ii) sensitive to X-rays but resistant to UV, and (iii) sensitive to both UV and X-rays.

VII. Atomic Energy of Canada Limited, Chalk River Nuclear Laboratories, Biology and Health Physics Division, Chalk River, Ontario, Canada. Communicated by Norman E. Gentner.

Summaries are provided below giving results and conclusions from recently completed research projects.

1) Repair in *Schizosaccharomyces pombe* as Measured by Recovery from Post-Irradiation Lethal Enhancement by Caffeine.
Norman E. Gentner and Mary M. Werner

The relatively high resistance of *S. pombe* wild-type to inactivation by uv- and ionizing radiation, and the isolation of radiation-sensitive mutants, implies that this eukaryotic organism possesses a definite capability for repair of radiation-induced damage. Neither the repair of DNA strand breaks or base damage caused by ionizing radiation, nor the excision of uv-induced pyrimidine dimers, has been demonstrated however. To obtain information on the time course of, and requirements for, the repair process in this organism, we developed the technique of following recovery by measuring the ability of irradiated cells to overcome the block caused by the presence of the dark repair inhibitor, caffeine. Caffeine, when present in the post-irradiation plating medium at a concentration which does not affect the viability of unirradiated cells, is known to reduce the survival of *S. pombe* after uv- or γ-irradiation. Uv- or γ-irradiated cells, if incubated in a complete medium before plating on caffeine-containing agar medium, were found to recover from susceptibility to this lethal enhancement by caffeine. This method was used to determine requirements for the repair process in *S. pombe*.

Post-irradiation protein synthesis appeared to be a requirement for repair of uv-induced damage but not for repair of γ-irradiation-induced damage. *S. pombe* 972h$^-$ cells, in which the incorporation of amino acids into protein had been inhibited by pre-incubation in cycloheximide-containing medium, displayed, after uv-irradiation and subsequent incubation, a low extent of recovery from caffeine inhibition, compared to cells in which protein synthesis had not been inhibited. After γ-irradiation, however, the recovery process occurred to the same extent in normal and cycloheximide-treated cells, although the rate was slightly slower in the latter. This suggested that the enzyme complement present during normal growth sufficed for repair of ionizing radiation damage, but that induction of new protein(s), presumably repair enzyme(s), was required for the excision of pyrimidine dimers.

Repair, as measured by recovery from caffeine inhibition, occurred in the absence of RNA and DNA synthesis after uv-irradiation; RNA and DNA synthesis was required, however, for recovery after γ-irradiation. These studies employed the adenine-requiring *S. pombe* strain 407h$^-$; the recovery process was followed by post-irradiation incubation in a minimal medium $\pm$ adenine supplement. Addition of adenine was not required for recovery from uv-damage, but was required for recovery from γ-ray-induced damage.

The degree of radiation sensitivity in different physiological states was found to be correlated with the rate of recovery from susceptibility to

post-irradiation lethal enhancement by caffeine. Cells of wild-type S. pombe are known to be more resistant to uv-inactivation in the logarithmic phase than during the stationary phase of growth; we have found that, correspondingly, the rate of recovery from caffeine inhibition after uv-irradiation was slower in stationary phase than in logarithmic phase cells. The opposite situation was found with respect to γ-irradiation - stationary phase cells were somewhat more resistant to inactivation by γ-rays than were logarithmic phase cells; similarly, the rate of recovery from caffeine inhibition was somewhat greater in stationary phase than in logarithmic phase cells. These results substantiate the thesis that the rate of recovery from caffeine inhibition is indeed measuring parameters related to the ability of cells to repair radiation-induced damage.

2) Repair of Radiation-induced Damage in Schizosaccharomyces pombe: Time Course of Recovery Between Split Doses and From Synergistic Interaction of uv- and γ-Irradiation.
Norman E. Gentner

We have found that a synergistic interaction exists with respect to inactivation of S. pombe wild-type by uv- and γ-irradiation. This is evidence for an overlap between repair processes acting on the two types of radiation damage; the capacity of cells to repair damage due to exposure to one type of radiation is decreased by prior exposure to the alternate type of radiation.

In the experiments described above on recovery of S. pombe from post-irradiation lethal enhancement by caffeine, it was noted that, for uv- and γ-ray doses which yielded equivalent survival, the recovery from caffeine inhibition after γ-irradiation proceeded somewhat more rapidly and completely than the recovery after uv-irradiation. The site of caffeine action is not known, and further, the point of the block by caffeine might not be the same for the repair processes acting after uv- or γ-irradiation. This technique gives information on the time course and requirements for repair only up to and including the step of caffeine inhibition; there might be additional requirements for complete repair, existing as a consequence of steps after the site of caffeine sensitivity, which would not be elucidated. In order to examine whether the irradiated and incubated cells which had overcome the caffeine block were completely repaired, and to examine overlap between the repair processes for the two types of radiation-induced damage, we applied two additional criteria which would have to have been satisfied if complete repair had occurred: when cells were given a uv- or γ-ray dose corresponding to 10% survival (a value past the shoulder of the inactivation curve and on the exponential death portion), and were incubated in complete medium, (1) did the incubated cells regain, for the same type of radiation, the shoulder on the inactivation curve characteristic of normal cells, and (2) was there a lessening, or elimination, of the degree of synergistic inactivation seen if a uv-exposure immediately followed a γ-ray exposure (or vice versa). Post-irradiation incubation times up to ten hours were employed; the post-irradiation division lag is such that no cell division occurred up to this time.

Following γ-irradiation to 10% survival and incubation sufficient to overcome the caffeine inhibitory effect (3 hours), the cells had largely regained the degree of resistance to γ-irradiation characteristic of normal cells, i.e., reconstruction of the shoulder of the γ-inactivation curve was evident. Similarly, after uv-irradiation to 10% survival, 3 hours incubation in complete medium (which sufficed for nearly the maximal attainable extent of recovery from caffeine inhibition), and subsequent exposure to various γ-ray doses, it was seen that the cells had largely recovered from the synergistic inactivation observed in the absence of the intervening

incubation period.

A considerably different effect was seen with respect to uv-inactivation curves. After uv-irradiation to 10% survival, no regaining of resistance (as exemplified by reconstruction of the shoulder) to subsequent uv-exposure was seen until after 7 hours of incubation. Even after 10 hours incubation, the cells were still more sensitive to inactivation by uv than normal cells; it is possible that full reconstruction does not occur until after cell division. A similarly long incubation period of γ-irradiated cells was necessary to overcome the synergistic inactivation to subsequent uv-exposure; in this case recovery was essentially complete by ten hours.

These experiments have provided information on the time course for complete recovery, and the overlap of systems for repair of uv- and γ-ray-induced damage.

After incubation sufficient to overcome the caffeine effect, cells surviving exposure to uv- or γ-irradiation were nearly as resistant to γ-irradiation as cells which had never been exposed to radiation. The site of caffeine inhibition in the repair pathway for γ-ray-induced damage may therefore be near the later steps of the sequence, since survivors in which repair had proceeded past the site of caffeine inhibition satisfied the criterion of complete repair.

Uv-irradiated cells, however, required an incubation period considerably longer than that required to overcome caffeine inhibition in order to regain some of the resistance to uv-irradiation characteristic of normal cells; this may indicate either (a) that the site of caffeine inhibition in the repair pathway for uv-damage occurred relatively early in the sequence, and hence longer incubation was required to effect complete repair, or (b) that the caffeine-sensitive pathway is involved in repair of only a portion of the uv-induced lesions, and a second, slower, caffeine-insensitive, pathway is also involved in repair of uv-induced damage. Also, although γ-irradiated cells regained their resistance to further γ-exposure relatively early, there persisted for a longer period some residual γ-ray damage which overlapped the repair pathway(s) for uv-damage.

Although the initial doses of uv- and γ-irradiation employed ultimately yielded the same survival, these experiments on shoulder reconstruction and overcoming of synergism indicate that S. pombe is more efficient at repairing γ-irradiation-induced damage than at repairing uv-irradiation-induced damage.

VIII. Institute of Physics, College of General Education, University of Tokyo, Komaba, Meguroku, Tokyo 153, Japan. Communicated by Takashi Ito.

1. Below follow the abstracts of two papers presented at the 15th Annual Meeting of the Japan Radiation Society, Kanazawa, Japan, 1972:

- - -

Mechanisms of Photosensitizing Action of Acridine Orange in Yeast. II. Different Effects of Two Wavelengths Absorbed by Acridine Orange-Nucleic Acid Complex. ITO, T.

Acridine orange (AO) applied to yeast cells is expected to bind to the nucleic acids in two states which characteristically absorb at 502 nm and 465 nm, respectively. The amount of AO uptake and its distribution were previously reported (J. Radiat. Res. 1972). The present experiments were performed to see if there is any difference between the two bound states in photosensitizing (photodynamic) actions. The results are summarized: (1) Dose-survival curves were different in shape. (2) Dose-frequency relationship for a genetic change (gene conversion at try locus) was linear, but more efficient at 510 nm than at 470 nm. (3) The frequency of gene con-

version at the same lethality was consistently higher for 510 nm than 470 nm. (4) A marked synergistic interaction between photodynamic and UV action was observed by the decrease of the shoulder in UV survival curves. Similar interaction was observed for gene conversion. Thus, it can be concluded that these two wavelengths do not activate the same reaction at the same site. The synergistic interaction would mean that the resulting photodynamic damage may have an inhibitory effect on the operation of a repair system if the large shoulder in UV survival curves is to reflect the recovering ability of the cell.

\- - -

Mechanisms of Photosensitizing Action of Acridine Orange in Yeast. III. Effects of Phosphotungstic Acid. KOBAYASHI, K. and ITO, T.

In every proposed scheme for photodynamic actions the rate of the initial photochemical process depends on the yield of the activated state of sensitizers. We measured, in the present experiments, the changes in the fluorescence yields and absorption spectra of acridine orange (AO) induced by the addition of phosphotungstic acid (PTA), an effective fluorescence quencher, in the presence of DNA or RNA. These changes were related to the amounts of sensitized photoinactivation of AO bound to yeast cells which had been similarly treated with PTA.

The following results were obtained: (1) The sensitized inactivation was suppressed roughly in proportion to the reduction of fluorescence of AO by the addition of PTA. (2) The addition of PTA at the time when AO molecules would have bound to nucleic acids showed no effect on the sensitized inactivation.

These results led us to a working hypothesis that AO must be in such a state to be photodynamically active that it can emit fluorescence, and that AO may be effective only when it binds to the nucleic acids.

These abstracts will appear in J. Radiat. Res. 14 (1973).

2. Summary of a manuscript recently submitted for publication is presented below:

Photodynamic Actions on Synchronous Cultures of Saccharomyces cerevisiae. ITO, T. and KOBAYASHI, K.

The sensitivity to the photodynamic inactivation mediated by acridine orange (AO) has been investigated at various cell stages of synchronous cultures of Saccharomyces cerevisiae. The reciprocals of D_{10} (dose at 10% survival) indicate that the sensitivity increases gradually from time zero cultures (nonbudding small cells selected from late logarithmic phase culture), and it reaches the highest sensitivity at the stage when the size of a bud approaches about two-thirds of the mother cell. Thus, the timing of the highest sensitivity is shifted toward a later stage from the so-called S stage of the DNA replication cycle. It was also suggested that, besides the stage-dependent sensitivity, there exists a sensitivity change according to the growth conditions of the culture.

3. A thesis has been submitted by K. Kobayashi for the Master's degree to the University of Tokyo.

Title: The role of the binding of acridine orange to the nucleic acids in photodynamic actions on yeast cells.

IX. Division of Genetics, National Institute of Radiological Sciences, Anagawa, Chiba, Japan. Communicated by Sayaka Nakai.

The following is a summary of a recent paper.

Induction and Repair of Gene Conversion in UV-Sensitive Mutants of Yeast.
Shigeyuki Mori and Sayaka Nakai.
Molec. gen. Genet. 117:187-196, 1972

SUMMARY

Photoreactivation effect on UV-induced allelic recombination has been examined using various combinations of leu 1 alleles in UV-sensitive and wild type diploid yeast, Saccharomyces cerevisiae. The frequencies of UV-induced heteroallelic reversion in UV-sensitive strains, presumably lacking dark-repair, are strikingly enhanced compared to those in wild type at the same doses under dark condition. However, these enhanced frequencies of reversion are diminished by photoreactivation almost to the level of those in wild type. The induced frequencies of homoallelic reversion (mutation) of relevant alleles are apparently lower than those of heteroallelic reversion. Phenotypic analysis for linked gene leu 1 on UV-induced heteroallelic revertants has shown that most of the revertants are of the nonreciprocal type recombination (mitotic gene conversion). These results would indicate that most of the dark-repairable damage leading to mitotic gene conversion after UV-light is due to pyrimidine dimers.

The following is an earlier publication from our laboratory:

Sayaka Nakai and Eiko Yamaguchi. Differential mutabilities to types of mutations with ultraviolet light between normal and UV-sensitive mutants of yeast. Japan. J. Genetics 44:355-365 (1969).

X. Van't Hoff Laboratorium, University of Utrecht, Sterrenbos 19, Utrecht, The Netherlands. Communicated by A. M. A. ten Berge.

A.M.A. ten Berge, G. Zoutewelle, D.H.J. Schamhart and K.W. van de Poll: We have been studying the regulation of maltose fermentation governed by MAL_6 in Saccharomyces carlsbergensis.

1. A.M.A. ten Berge: Genes for the fermentation of maltose and α-methylglucoside in Saccharomyces carlsbergensis. Molec. gen. Genet. 115, 80-88 (1972).

2. A.M.A. ten Berge, G. Zoutewelle and K.W. van de Poll: Regulation of maltose fermentation in Saccharomyces carlsbergensis. I. The function of the gene MAL_6, as recognized by mal_6-mutants. Molec. gen. Genet. (1973), in press.

   In this paper it is argued that the gene MAL_6, as recognized by a number of mal_6-alleles and mal_6-ts-alleles, is neither a structural gene for maltase nor for maltose-permease but that it has to be considered as a positive regulatory gene.

3. A.M.A. ten Berge, G. Zoutewelle, K.W. van de Poll and H.P.J. Bloemers: Regulation of maltose fermentation in Saccharomyces carlsbergensis. II. Properties of a constitutive MAL_6-mutant. Molec. gen. Genet., submitted for publication.

   In this paper a constitutive mutant (C_2), closely linked to MAL_6, is described, which is different from the constitutive MAL_4-allele, described by Khan and Eaton, Molec. gen. Genet. 112, 317-322 (1971), in being recessive, catabolite repression sensitive and not superinducible by maltose. Moreover maltase produced by C_2 is not different from wild type maltase. The most intriguing property of C_2 is, that in hybrids of C_2 with the mal_6-mutants the inducibility is restored.

4. Other constitutive MAL_6-mutants.
   In collaboration with R. Needleman, Brooklyn College, New York, 12 other constitutive MAL_6-mutants have been isolated. One of them is dominant in

hybrids with the wild type strain as well as in hybrids with mal_6-mutants; the others are recessive to the wild type but among hybrids with mal_6-mutants a variation is found between complete inducibility to constitutivity. These results indicate an interaction between the products of the recessive constitutive alleles and the mal_6-alleles.

5. An allele-nonspecific suppressor of constitutive MAL_6-mutants.
A recessive suppressor has been isolated, which changes 8 constitutive MAL_6-mutants tested into a maltose negative strain. The suppressor (called mal_x) is not linked to MAL_6 and has no effect on the wild type MAL_6-gene. Since strains homozygous for mal_x but heterozygous for a constitutive allele and a mal_6-allele do not grow on maltose, the possibility is offered for an establishment of map distances between constitutive and mal_6-alleles by mitotic recombination (pers. comm. R. Needleman).

6. A non-mendelian determinant of the lagtime to maltose in petite strains.
We found that petites of S. carlsbergensis start growth on maltose-plates immediately, whereas petites of some strains of S. cerevisiae show a lagtime longer than a week. If a strain with the short lagtime is crossed with a strain having the long lagtime, the hybrid has the long lagtime; moreover all spores from that hybrid have the long lagtime, although the length of that lagtime varies among different spores. One possibility to explain these data is the assumption that the parent having the long lagtime contains a number of dominant genes, each affecting the adaptation to maltose, for which the parent with the short lagtime is negative. A second possibility is that the determinant of the lagtime is inherited cytoplasmically like argued for the dominant ure-3 factor by Lacroute (J. of Bacteriol. 106, 519-522 (1971)); in that case it is suggested, that our determinant is not mitochondrial, since no mitotic segregation was observed.

7. A catabolite repression insensitive mutant.
A spontaneous mutant, suppressing the phaenotype of the long lagtime of maltose-growth in ρ^--strains, was found which is insensitive to catabolite repression for maltase, for α-methylglucosidase, invertase, galactase-1-P-uridyl-transferase and succinic dehydrogenase. The ratio between the enzyme-levels of mutant and wild type after growth on glucose is respectively 130, 110, 20, 9 and 3 for the enzymes mentioned. The insensitivity to catabolite repression segregates together with flakiness of the cells. This catabolite repression insensitive mutation (flk) is not linked to MAL_6 and also not centromere linked, in contrast with which was mentioned earlier (A.M.A. ten Berge: Genen en Phaenen 15, 27-28 (1972)).
A strain which carries a mal_6-mutation together with the flk-mutation does not grow on maltose although the cells have a high derepressed level of maltase. This proves again that the mal_6-mutants are not structural mutants for maltase.
The level of cyAMP in the flk-mutant is under current investigation.

The topics described under numbers 4, 5, 6 and 7 will be dealt with in the Ph.D. Thesis of A.M.A. ten Berge, to be published in October 1973, and will also be published in current journals.

XI. Brooklyn College of the City University of New York, Department of Biology, Brooklyn, New York 11210, U.S.A. Communicated by Norman R. Eaton.

Summaries of two papers which have been accepted for publication in Molecular General Genetics follow.

1. Zimmermann, F. K., N. A. Khan and N. R. Eaton: Identification of New Genes Involved in Disaccharide Fermentation in Yeast.

SUMMARY

Maltose non-fermenting mutants were obtained from strains carrying a MAL4 allele which permits constitutive synthesis of maltase. Cells carrying this allele are able to utilize sucrose in the absence of the "classical" sucrose genes. All maltose non-fermenting mutants were also sucrose non-fermenters. Eight mutants had become maltase negative; 19 mutants could still form maltase constitutively, but could no longer ferment maltose.

In crosses with segregational maltose and sucrose non-fermenting strains, maltase negative mutants gave diploids unable to ferment maltose and sucrose. Maltase positive, non-fermenting mutants gave diploids which readily fermented maltose and sucrose. This latter type of mutants was designated dsf (disaccharide fermentation) mutants.

The diploids derived from crossing non-fermenting mutants with segregational non-fermenters were subjected to tetrad analysis. Maltase negative non-fermenters gave only non-fermenting progeny. The dsf mutants segregated both maltose-fermenting and non-fermenting progeny, some of which showed the dsf phenotype. This indicated that none of the dsf mutants had a defect in a gene closely linked to MAL4. Crosses between dsf mutants and strains carrying the maltose genes MAL2 and MAL3 showed that the mutations affected maltose fermentation in general. Sucrose fermentation in the presence of the "classical" sucrose gene SUC3 was not affected, nor were fermentation of glucose, fructose and galactose.

The uptake of radioactivity from uniformly labeled maltose appeared to be blocked in mutants of at least four of the dsf genes. Only one non-leaky and a leaky mutant showed a significant uptake.

These results suggest that there is an extremely complex transport system for maltose and sucrose or that the utilization of these disaccharides requires a complex series of metabolic reactions.

2. Khan, Nasim, F. K. Zimmerman and N. R. Eaton: Genetic and Biochemical Evidence of Sucrose Fermentation by Maltase in Yeast.

SUMMARY

Strain 1403-7A, which carries the MAL4 gene responsible for constitutive maltase synthesis, can ferment sucrose in the absence of sucrose genes. Sucrose fermentation cannot be separated from maltose fermentation either by genetic recombination or by mutation. Crude extracts of strain 1403-7A also lack the classical invertase, and fractionation of such extracts by gel filtration results in a peak of maltase activity which corresponds exactly to the activity with respect to sucrose hydrolysis. Moreover, *in vitro*, both of these disaccharides are hydrolyzed maximally at pH 6.4 to 6.8. It is suggested that, as long as sucrose can penetrate the cell, maltase, if present at high level in any strain, should be able to hydrolyze sucrose and therefore permit its fermentation. We have, however, identified in one of our yeast stocks a single recessive gene (ssf gene) which specifically interferes with sucrose fermentation in strain 1403-7A, probably by limiting the penetration of sucrose.

XII. Fachbereich Biologie Technische Hochschule, 9 Schnittspahnstr., 61 Darmstadt, Germany. Communicated by F. K. Zimmermann.

MUTATIONAL SUCROSE NON-FERMENTERS

Sucrose fermentation in *Saccharomyces cerevisiae* has been shown to proceed in the presence of any one of six unlinked genes called SUC (sucrose) genes. In the presence of a SUC gene sucrose fermentation is mediated by invertase. However, sucrose fermentation can be mediated by maltase as well if maltase synthesis is constitutive as in the presence of the maltose gene MAL4 (Khan, Zimmermann

and Eaton, Molec. Gen. Genet., in press). Sucrose non-fermenters can be obtained as segregants from crosses between strains carrying different SUC genes, they are called segregational sucrose non-fermenters. The true nature of the presently established SUC genes is unknown, they could be structural, regulatory or indispensable, modifying genes. In order to gain some access to this problem, mutational sucrose non-fermenters were isolated after UV treatment from the haploid strain EK-6B carrying the gene SUC3 in tight linkage with the maltose gene MAL3. Four mutants were isolated and designated suc3-1, suc3-2 (no sucrose fermentation at all), suc 3-3 and suc3-2o (very slow sucrose fermentation). Maltose fermentation was not affected in these mutants. They were then crossed to a segregational sucrose and maltose non-fermenter. All diploids fermented maltose but not or only slowly sucrose. Tetrad analysis revealed that maltose fermentation segregated normally 2:2. No wild type-like sucrose fermenters were observed among the segregants, but in crosses involving suc3-3 and suc3-2o the very slow fermentation segregated in a 2:2 ratio.

In these latter crosses all maltose fermenters were very slow sucrose fermenters and vice versa. This indicates that the induced mutations had affected a genetic region closely linked to MAL3 and probably identical with SUC3. This is in sharp contrast to the situation with mutational maltose non-fermenters which can be caused by defects in at least eight different genes (Zimmermann, Khan and Eaton, Molec. Gen. Genet., in press).

- - -

A CONVENIENT STRAIN FOR VISUAL DEMONSTRATION OF MITOTIC CROSSING OVER

The unambiguous demonstration of mitotic crossing over depends on the detection of the two reciprocal products generated by a crossing over event. This requires a diploid which is heterozygous for two recessive alleles which are located on the same chromosome arm but arranged in repulsion, i.e., in this particular case, they have to be situated on different chromosomes of a homologous pair. Moreover, due to the recessiveness of the alleles under consideration and the heterozygous condition such a diploid will express a dominant phenotype. Mitotic crossing over between the centromere and the proximal recessive allele location will lead, at a 50% chance, to a segregation in the subsequent mitotic cell division of a daughter cell homozygous for one of the recessive alleles, whereas the other daughter cell will be homozygous for the other allele. Two clones expressing the recessive phenotypes will arise from such a cell. In tissues of Drosophila, this will create the classical Sternian twin spots; in yeast, this will lead to a colony, half of which expresses one of the recessive traits, and the other half the other trait. Such twin spotted colonies are easy to recognize if the two recessive alleles cause pigmentation of the colonies different from the pigmentation of those colonies made up of cells of the original heterozygous genotype. Mutant alleles of the gene locus ade2 cause the accumulation of a red pigment and are recessive to wild type. Out of a total of 132 newly induced ade2 mutant alleles two were selected: ade2-40 is a completely blocked allele which causes the typically red pigmentation whereas allele ade2-119 is a leaky allele which causes only a limited amount of pigment accumulation to the effect that colonies are only pink. On top of that, the two alleles complement each other to give a heteroallelic diploid which forms white colonies. Mitotic crossing over creates colonies with a red and a pink sector in response to treatment with ultraviolet light, ethylmethanesulfonate and nitrous acid. This diploid strain, D5, is available for distribution.

XIII. Institut für Biochemie, Universität Würzburg, 11 Röntgenring, 87 Würzburg, Germany. Communicated by Eckhart Schweizer.

The following is a summary of an article recently submitted for publication.

Pantothenate Free Mutants of the Yeast Fatty Acid Synthetase Complex. E. Schweizer, B. Kniep, H. Castorph and U. Holzner.

Among 44 Saccharomyces cerevisiae fatty acid synthetase (fas) mutants screened for their ability to incorporate C^{14}-labeled pantothenic acid into the fatty acid synthetase multienzyme complex, especially those of fas-complementation group VII apparently had lost this ability. The purified fatty acid synthetase complexes of all the 23 fas-mutants available from this group were shown to be free of C^{14}-labeled pantothenic acid. The amount of pantothenate incorporated into the enzymes of several other fas-mutants of the non-pleiotropic complementation groups II, V, VI and VIII, however, was similar to that observed with wild type fatty acid synthetase. The purified pantothenate-free fatty acid synthetases of five different fas-mutants have been tested for the seven known component enzymes of the complex. In all mutants, only the β-ketoacid synthetase was inactive, whereas in vitro all the other partial activities were unimpaired. By SDS polyacrylamidgel electrophoresis, no differences could be observed between the protein structure of the pantothenate-free mutant fatty acid synthetase and that of the wild type complex. Both were separated into three different components A, B and C with molecular weights of 185 000, 180 000 and 177 000, respectively. However, some fas-mutants consist only of the components A and B, others only of B and C. The study of various C^{14}-pantothenate labeled mutant fatty acid synthetases suggested that component C originates from A, presumably by limited proteolysis. In the undegraded complex AB, C^{14}-pantothenate is only associated with the component A. Since in some mutants A is completely converted to C, it is concluded that A is one distinct component rather than a group of components with identical molecular weights. It is tentatively suggested that the gene product of fas 2 - one of the two known fatty acid synthetase gene clusters in yeast - is only a single multifunctional polypeptide chain. Therefore, it appears that in yeast the "Acyl Carrier Protein" is not an individual protein component of the fatty acid synthetase complex, but only a distinct region of the multifunctional polypeptide chain encoded by fas 2.

XIV. Albert Einstein College of Medicine of Yeshiva University, Developmental Biology and Cancer, 1300 Morris Park Avenue, Bronx, New York 10461, U.S.A. Communicated by Reed Wickner.

The following is the summary of an article recently submitted for publication.

Mutants of S. cerevisiae that incorporate dTMP into DNA in vivo. Reed B. Wickner.

Spontaneous mutants of Saccharomyces cerevisiae able to incorporate dTMP into DNA have been selected based on their ability to grow in the presence of aminopterin and sulfanilamide if dTMP is present. Essentially all mutants (called tup) selected in this way required dTMP for growth in the presence of the two drugs but none required dTMP in the absence of the drugs. Neither thymine nor thymidine would satisfy this requirement.

Equimolar amounts of P^{32} and H^3-base-labeled dTMP were incorporated by the mutants into alkali stable, DNase-sensitive material. In the presence of drugs, this incorporation was sufficient to account for most, if not all, of the thymine residues in the cellular DNA, while in the absence of the drugs, only about 30% of the thymine residues originated from the medium. Mutants derived from most strains required 100 μg/ml of dTMP to achieve maximal growth in the presence of the drugs or maximal DNA labeling. This is partly explained by the substantial breakdown of dTMP during growth. tup mutants derived from a phosphatase-deficient strain (J. Kuhn and R. Snow) had a 10-fold lower dTMP requirement for growth on solid media in the presence of drugs.

Of 29 mutants examined all were recessive and 17 showed 2:2 segregation in crosses with a wild-type strain. These fell into 4 complementation groups, one

(tup-1) mapped on chromosome III, another (tup-3) mapped on chromosome II and a third (tup-4) was centromere-linked. Strains of the genotype α tup-1 mated with lower than normal efficiency with a strains, but with higher than normal efficiency with α strains. Strains of genotype a/α tup-1/tup-1 failed to sporulate while homozygous diploids for tup 2, tup 3 or tup 4 sporulated normally, as did a/α tup 1/ + strains.

XV. Laboratoire d'Enzymologie, C.N.R.S., 91190-Gif-Sur-Yvette, France. Communicated by H. de Robichon-Szulmajster.

1. Biosynthesis of Methionine and Its Control in Wild Type and Regulatory Mutants of Saccharomyces cerevisiae
J. Antoniewski and H. de Robichon-Szulmajster
(will appear in the June issue of "Biochimie")

The overall biosynthesis of methionine has been examined under different growth conditions and in different strains, including regulatory mutants. The following points can be stressed:

1) The pool size of free methionine remains remarkably constant during the exponential phase of growth in wild type strains as well as in methionine overproducers (see below).

2) Methionine accumulates in the pool of some regulatory mutants, the most extreme one, bearing the mutation eth2-2, having a 20-fold higher methionine pool size than its corresponding wild type.

3) Accumulation is accounted for by increased rates of synthesis observed in these regulatory mutants.

4) A part of the methionine overproduced is excreted into the medium. A part of it is probably metabolized further.

5) Under conditions of either methionine-mediated repression or S-adenosyl methionine-mediated repression, kinetics of decrease of methionine biosynthesized are compatible with repression being the major regulatory mechanism for this biosynthetic pathway in Sacch. cerevisiae.

2. Effects of Regulatory Mutations upon Methionine Biosynthesis in Saccharomyces cerevisiae: LOCI eth2 - eth3 - eth10.
H. Cherest, Y. Surdin-Kerjan, J. Antoniewski and H. de Robichon-Szulmajster
(submitted to J. Bacteriol.)

The effects of mutations occurring at three independent loci, eth2, eth3 and eth10, have been studied on the basis of different criteria: level of resistance towards two methionine analogues (ethionine and selenomethionine), pool sizes of free methionine and S-adenosyl methionine (SAM) under different growth conditions and susceptibility towards methionine-mediated repression and SAM-mediated repression of some enzymes involved in methionine biosynthesis (met group I enzymes). It has been shown that: 1) the level of resistance towards both methionine analogues roughly correlates with the amount of methionine accumulated in the pool, 2) the repressibility of met group I enzymes by exogenous methionine is either abolished or greatly lowered, depending upon the mutation studied, 3) the repressibility of the same enzymes by exogenous SAM remains, in at least three mutants studied, close to that observed in a wild type strain, 4) the accumulation of SAM does not occur in the most extreme mutants either from endogenously overproduced or from exogenously supplied methionine, 5) the two methionine activating enzymes, methionyl-tRNA synthetase and methionine adenosyl transferase, do not seem modified in any of the mutants presented here, 6) the amount of $tRNA^{met}$ and its level of charge are alike in all strains.

Thus, the three recessive mutations presented here affect methionine-mediated repression, both at the level of the overall methionine biosyn-

thesis which results in its accumulation in the pool, and at the level of the synthesis of met group I enzymes. Implications of these findings have been discussed.

XVI. Department of Genetics, Institut National Agronomique, 16 Rue Claude-Bernard, Paris 5°, France. Communicated by H. Heslot.

1. The following paper has been accepted for publication in "Archiv für Microbiologie".

A study of conjugation, sporulation and meiotic segregation in *Candida lipolytica*.
C. M. Gaillardin, V. Charoy and H. Heslot.

Summary: We have attempted to optimize conjugation and sporulation in *Candida lipolytica*, by concentrating on conditions of culture and growth.

Copulation between compatible strains is a rare event, particularly in the case of auxotrophic mutants. However, diploids can be selected for on minimal medium provided the parents are suitable auxotrophs. These diploids can multiply vegetatively for many generations. They can also be induced to sporulate at a very high frequency.

Free ascospores were isolated by means of paraffin oil and segregations of markers could be studied. At first quite irregular, these segregations improved following a number of brother x sister matings. At the same time, the mean number of spores per ascus as well as spore germinability were considerably increased.

2. The following paper has been accepted for publication in Biochim. Biophys. Acta.

Regulation of the biosynthesis of purine nucleotides in *Schizosaccharomyces pombe*. III. Kinetic studies of adenylosuccinate synthetase.
M. Nagy, M. Djembo-Taty and H. Heslot.

Summary: High levels of adenylosuccinate synthetase have been found in the yeast *Schizosaccharomyces pombe* and the enzyme was partially purified from an ade-8 mutant, lacking succino-AMP lyase.

The enzyme is strongly inhibited by AMP and GMP. Whereas GMP seems to act by a direct competition for the GTP binding site, AMP appears as an allosteric effector, showing at nonsaturating substrate concentrations a homotropic effect as well as a heterotropic effect upon the GTP and aspartate binding.

The initial rate experiments indicate a fully random mechanism of substrate binding.

XVII. Department of Food Science and Technology, University of California, Davis, Ca. 95616, U.S.A. Communicated by M. W. Miller.

Below follow abstracts of two publications. The first one appeared in the April issue of the Journal of Bacteriology and the second one will appear in the July issue.

1) Electron Micrography of Bud Formation in *Metschnikowia krissii*.
L. T. Talens, Mary Miranda and M. W. Miller.

The fine structure of bud formation of *Metschnikowia krissii* was studied by means of ultramicrotomy and transmission electron microscopy. Bud protrusion and development were observed by scanning electron microscopy. Bud formation in this yeast takes place by an extension of a small localized area of the existing parent wall. The parent cell and its bud are initially separated by the plasmalemma, creating an intercellular site

within which the generation of new cell wall (bud and birth scar areas) occurs centripetally. When the dividing wall is complete and new cell wall material is formed, a narrow cleavage plane becomes increasingly defined. This cleavage plane apparently proceeds laterally toward the direction of the existing outer walls which rupture, resulting in the separation of the bud from the parent cell. The bud scar is prominently convex in shape; the birth scar is less conspicuous and initially concave in shape. Comparison of bud formation in M. krissii is made with that observed in Saccharomyces cerevisiae and Rhodotorula glutinis.

2) Electron Micrographic Study of the Asci and Ascospores of Metschnikowia Kamienski.
L. T. Talens, M. W. Miller and Mary Miranda.

Internal and surface structures of asci and ascospores were studied by transmission electron microscopy (TEM) and by scanning electron microscopy (SEM) to establish the character and number of ascospores within the ascus of Metschnikowia krissii. Enzyme digestion with snail gut enzymes and SEM examination suggested the presence of a single ascospore enclosed in a thick sheath of epiplasmic materials. Two closely associated ascospores without an epiplasmic sheath were clearly distinguishable from asci of M. bicuspidata var. chathamia when similarly treated. Ultramicrotomy and TEM established conclusively that M. krissii produced a single ascospore per ascus. Neither SEM nor TEM revealed any morphological detail of the ascospores of taxonomic significance.

XVIII. Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02154, U.S.A. Communicated by Dr. Harlyn O. Halvorson.

Dr. Martin Kuenzi and Dr. Marjorie Tingle have recently observed that in Saccharomyces cerevisiae sporulation and germination can occur in cultures devoid of detectable mitochondrial DNA. Such "genotypically petite" cultures were prepared by treatment of respiratory competent cells with ethidium bromide prior to transfer to sporulation medium. The kinetics and extent of ascospore formation was similar to that observed in control cultures. The spores produced were both genotypically and phenotypically petite but no marked differences were observed in the germination and outgrowth compared to wild-type spores.

Dr. Isamu Takano from Suntory Limited, Osaka, Japan, has recently joined our group.

Recent publications:

M. Tingle, A. J. Singh Klar, S. A. Henry and H. O. Halvorson. Ascospore formation in yeast. In: Microbial Differentiation. 23rd Symposium of the Soc. for General Microbiology. London, April 1973, pg 209. (Cambridge Univ. Press 1973).

M. Tingle and H. Halverson. 1972. Biochemical and genetic characterization of β-glucosidase mutants in Saccharomyces lactis. J. Bacteriol. 110:196-201.

M. Tingle and H. Halverson. 1972. Mutants in Saccharomyces lactis controlling both β-glucosidase and β-galactosidase activities. Genet. Res., Cambr. 19:27-32.

XIX. Department of Food Science and Technology, University of California, Davis, Ca. 95616, U.S.A. Communicated by A. Sommer.

Below follows the abstract of the Ph.D. dissertation of Andreas Sommer. The work was done under the guidance of Professor M. J. Lewis.

Biochemical Studies of the Spheroplast Membrane from Saccharomyces carlsbergensis.

The plasma membrane of Saccharomyces carlsbergensis was isolated by lysis of spheroplasts and purified by means of a discontinuous sucrose gradient.

Extraction of the isolated membranes with dilute buffer released 80% of the total membrane protein as a macromolecular complex, leaving the remaining 20% of protein as a highly water insoluble aggregate.

Solubilization of the isolated membranes by sodium dodecyl sulfate and subsequent gel filtration on Sepharose 6B and Sephadex G-200 indicated that the majority of the membrane phospholipid is dissociated from the membrane protein, which suggests the existence of extensive hydrophobic bonding in the intact membrane. A small membrane fraction appeared as lipoprotein upon gel filtration; the origin of this structure has not yet been verified. This lipoprotein fraction, however, contained the proteins which specifically bind calcium and magnesium, while potassium was found to be loosely associated with all membrane proteins.

Upon removal of the sodium dodecyl sulfate, the lipoprotein-containing fraction was found to be the only membrane component reaggregating to visible particles.

Polyacrylamide gel electrophoresis of the sodium dodecyl sulfate solubilized membrane proteins revealed that the yeast plasmalemma contains at least 19 polypeptides of molecular weights ranging from 170,000 to less than 14,000 with the majority of the membrane protein having a MW of 14,000 to 33,000.

The buffer insoluble membrane fraction was found to consist of predominantly higher molecular weight polypeptides (MW 40,000 to 60,000).

XX. Department of Food Science and Technology, University of California, Davis, Ca. 95616, U.S.A. Communicated by H. J. Phaff.

The following is a summary of the Ph.D. Dissertation of Graham H. Fleet. The research was performed under the guidance of Professor H. J. Phaff.

β-Glucanases of yeasts

The work reported here is a continuation of earlier studies in this laboratory on the occurrence and function of exo-β-glucanases in multilaterally-budding yeasts and an endo-β-1,3-glucanase associated with two bipolarly-budding species of Hanseniaspora. In the present work a study was made of the association of glucanases with species of the fission yeast genus Schizosaccharomyces. An additional reason for considering this genus was the presence of high levels of α-1,3-linked glucan in the cell walls of its species.

Cell-free extracts and washed cell wall preparations of the four species, S. pombe, S. versatilis, S. malidevorans and S. octosporus were screened for the presence of β-1,3, β-1,6, and α-1,3-glucanase activities. The cell-free extracts and wall preparations of all of the species exhibited measurable amounts of β-1,3-glucanase activity, but the preparations from S. versatilis showed by far the highest levels of activity. Low levels of β-1,6-glucanase activity were found in all of the cell-free extracts, but the presence of this activity in cell wall fractions could not be conclusively demonstrated. No evidence for the occurrence of α-1,3-glucanase in any of the species or fractions was obtained. In addition to the high levels of β-1,3-glucanase activity in cell extracts and wall preparations of S. versatilis β-1,3 glucanase is also excreted into the culture medium. For these reasons this species was chosen for a detailed study. It was found that the β-1,3-glucanases in the cell extracts and extracellular culture medium were exo-enzymes, while the wall-associated β-1,3-glucanase demonstrated formation of oligosaccharide intermediates during β-1,3 glucan hydrolysis, suggesting that an endo-β-glucanase was present.

Using a combination of Sephadex G-100 and DEAE-cellulose chromatography the exo-β-1,3-glucanases from the intracellular extracts and the culture fluid of

S. versatilis were extensively purified and their properties determined. The intracellular and extracellular enzymes exhibited similar properties. The exo-pattern of hydrolysis of the purified enzyme was confirmed; it had a molecular weight of approximately 43,000; and it was found to hydrolyze both laminarin and pustulan, the latter slowly (Km, 6.25 mg/ml, laminarin; Km, 166.6 mg/ml, pustulan). Evidence was obtained supporting the view that a single protein is responsible for both hydrolytic activities as is known to be the case for the exo-β-glucanases of S. cerevisiae, H. anomala and K. fragilis. The purified exo-β-glucanase showed negligible hydrolytic activity on isolated cell walls from S. pombe or baker's yeast. However, alkali extracted walls of S. pombe underwent enzymic digestion, indicating that in untreated cell walls the non-reducing terminals of the β-glucan in the wall may be inaccessible to the enzyme.

Isolated washed cell walls of S. versatilis exhibited the phenomenon of autohydrolysis. When these walls were suspended in sodium succinate buffer at pH 5.0 and incubated, a substantial release or solubilization of oligosaccharides and glucose occurred. If the walls were incubated in the presence of laminarin a more rapid release of reducing groups was noted. Chromatographic analyses of the products of these reactions revealed β-1,3-linked oligosaccharides as well as glucose. These observations suggested the presence of an endo-β-1,3-glucanase in cell wall preparations of S. versatilis. During cell wall autohydrolysis the wall-associated glucanase was slowly solubilized. Following autolysis on a large scale the solubilized material was applied to a column of DEAE-cellulose equilibrated with 0.01 M sodium succinate buffer, pH 5.0. This procedure resolved the β-1,3-glucanase activity in the crude autolysate into two active fractions, one of which did not adsorb to the DEAE-cellulose and was found to be an endo-β-1,3-glucanase. This activity represented approximately 13% of the total glucanase activity applied to the column. The other activity remained bound to the column and after elution with a linear gradient of NaCl was found to be an exo-β-1,3-glucanase. Subsequent purification and analyses showed this latter enzyme to be identical to the exo-β-glucanase described above. The endo-β-1,3-glucanase was further purified by a combination of carboxymethyl-cellulose and Sephadex G-100 chromatography. The purified enzyme was specific for the β-1,3-glucosidic linkage and exhibited a high affinity for laminarin (Km = 0.33 mg/ml). The enzyme did not hydrolyze pustulan or laminaribiose. Based on its electrophoretic mobility in SDS polyacrylamide gels the enzyme was assigned a molecular weight of approximately 97,000. In contrast to the exo-β-glucanase, the endo β-1,3-glucanase showed high lytic activity towards native cell walls of S. pombe, but lower activity towards cell walls of baker's yeast.

Cell wall preparations from S. cerevisiae, S. rosei, K. fragilis, H. anomala, P. pastoris and C. utilis were examined for the presence of β-1,3-glucanase activity. Cell walls from all of the species tested exhibited the property of autohydrolysis and possessed various levels of β-1,3-glucanase, part of which was endo-activity. Cell wall-associated β-1,3-glucanases are probably of universal occurrence in yeasts where β-1,3-glucan is the rigid, structural wall component.

Various bacterial glucanases have been purified to a degree of purity exceeding that previously obtained in our laboratory (J. Bacteriol. 89:1570, 1965). The results of this work will be reported in the fall issue of the Yeast News Letter.

Some of the work reported above is currently in press in the Proceedings of the Third International Symposium on Yeast Protoplasts, Salamanca, Spain in October 1972 (Academic Press, London).

Dr. Fleet will be spending a two-year postdoctoral period in the laboratory of Professor David Manners, Department of Brewing and Biological Sciences,

Heriot-Watt University, Edinburgh, Scotland.

XXI. Université de Montpellier, Faculté de Pharmacy, 2 Av. Charles Flahault, Montpellier, France. Communicated by J. M. Bastide.

The following is a brief abstract of the doctoral dissertation of Dr. Pédro Travé. The work was done under the guidance of Professor J. M. Bastide.

Etude de la paroi de 46 espèces de *Candida* par dégradation enzymatique.

Abstract

Protoplasts were obtained from the yeast *Candida macedoniensis* by the use of a reducing agent (thiols or sodium sulphide) and a glucanolytic enzyme (*Helix pomatia* juice or exo-β-(1-3)-D-glucanase from Basidiomycete QM806). A proteolytic enzyme could be substituted for the reducing agent. Culture of the yeast on a colchicine-containing medium made it possible to omit the reducing agent in the preparation of protoplasts.

The reducing agents, or the proteolytic enzymes, always act before β-(1-3)-D-glucanase. It would thus seem likely that the rigid structure of the cell wall is essentially due to a protein, containing multiple disulphide bonds, and a framework of β-(1-3)-D-glucans. In addition, the early activity of the reducing agents suggests the possibility of a superficial location for the protein, which would thus cover the glucan framework.

Then, forty six species of *Candida* were studied either by the joined lytic action of a reducing agent (2-mercaptoaethylamin-HCl) and an enzyme (purified exo-β-(1-3)-D-glucanase) or the separate action of these two agents, one acting before the other. We could separate two groups of *Candida*:

- The yeasts which yielded protoplasts are classified in a first group. This result was obtained either by the joined action of both elements or by the previous action of the reducing agent. These yeasts of the first group do not release urease, and attack glucose anaerobically. In the first group are the following species: *C. albicans*, *C. atmosphaerica*, *C. berthetii*, *C. boidinii*, *C. brumptii*, *C. castellanii*, *C. claussenii*, *C. cloacae*, *C. fabiani*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*, *C. macedoniensis*, *C. maltosa*, *C. melinii*, *C. membranefaciens*, *C. obtusa*, *C. oregonensis*, *C. parakrusei*, *C. parapsilosis*, *C. pelliculosa*, *C. pseudotropicalis*, *C. pulcherrima*, *C. rhagii*, *C. robusta*, *C. rugosa*, *C. silvae*, *C. silvicola*, *C. solani*, *C. stellatoidea*, *C. tenuis*, *C. tropicalis*, *C. truncata*, *C. utilis*, *C. viswanathii*, *C. zeylanoides*.

The yeasts which do not yield protoplasts are in the second group. These yeasts hydrolyse urea and do not attack glucose. There are: *C. aquatica*, *C. bogoriensis*, *C. curvata*, *C. diffluens*, *C. frigida*, *C. gelida*, *C. humicola*, *C. japonica*, *C. lipolytica*, *C. scottii*.

These results seem to demonstrate that among the *Candida* the cell wall structure may be an important taxonomic criterium.

XXII. Departamento de Microbiología, Facultad de Ciencias y C.S.I.C. Universidad de Salamanca, Spain. Communicated by J. R. Villanueva.

J. M. Sierra, has presented his Doctoral Thesis entitled "Studies on enzymic lysis and biosynthesis of yeast cell walls". This dissertation, has been directed by Prof. J. R. Villanueva and Dr. R. Sentandreu and has covered some aspects about the enzymatic characterization of the lytic system produced by *Streptomyces venezuelae RA* as a first step towards understanding its mechanism of action on yeast cell walls. β-(1,3) glucanase, β-(1,6) glucanase and chitinase were detected in the enzymatic system. It was verified that protoplasts can be obtained only of those moulds and yeasts in which the cell wall consists of glucan-chitin, glucan-mannan and glucan-chitin-mannan. These

results suggest that the lytic activity of this enzymatic system must be mainly due to the joint action of three activities. For separating and characterizing these activities the classical methods of fractional precipitation with ammonium sulphate and gel filtration have been used. Elution through exchange columns using NaCl as the gradient allows a very good separation of β-(1,3) glucanase and β-(1,6) glucanase.

The biosynthesis of mannan and glucan, the two main components of Sacch. cerevisiae cell walls has been investigated in synchronous cultures prepared by two different methods. The first one is based on the findings by several researchers that the yeast cell undergoes periodic fluctuations in density during the cell cycle. The second method makes use of a mutant defective in a gene function needed at specific stages of the cell division cycle. Cells cynchronized by either system showed that DNA replication is periodic and takes place at a specific point of the cell cycle. RNA, glucan and mannan synthesis by contrast, occurred at a constant rate throughout the entire cell cycle. These findings indicate that either the genes involved in the synthesis of the RNA messenger of the glucan and mannan synthetases and/or the mannoproteins are always available for transcription and/or these RNA messenger and/or the glucan and mannan synthetases have a slow decay. Other possibility may be related to the gene dosage.

Dr. S. Gascón has been appointed Professor Agregado of Biochemistry of the Faculty of Medicine of the University of Oviedo.

XXIII. Yeast Group of the Department of Technical Microbiology, Institute of Microbiology of the Czechoslovak Academy of Sciences, Prague 4, Budějovická 1083, CSSR. Communicated by Dr. K. Beran.

Below follow abstracts of works that were carried out partly by our group and partly in cooperation with other groups and that were published or have been submitted for publication.

1) The Chitin-Glucan Complex in Saccharomyces cerevisiae I. IR and X-Ray Observations.
K. Beran, Z. Holan and J. Baldrián.
Department of Technical Microbiology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague 4, and Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague 6.
Abstract: The content of glucosamine in the walls of daughter cells (without bud scars) and mother (multiscar) cells of Saccharomyces cerevisiae was examined in a control and after treatment with dilute alkali, acid and buffer. The occurrence of chitin in the bud and birth scars is discussed. The results of IR and X-ray analysis of cell-wall fractions indicate the presence of alfa-chitin which is a part of the chitin-glucan complex. The size of the crystallite of alfa-chitin in this complex is about 60 Å.

Folia microbiologica 17:322, 1972.

2) The Chitin-Glucan of Saccharomyces cerevisiae II. Localization of the Complex in the Encircling Region of the Bud Scar.
O. Seichertová, K. Beran, Z. Holan and V. Pokorný.
Department of Technical Microbiology and Department of General Microbiology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.
Abstract: Fractions of the cell wall of Saccharomyces cerevisiae where a chitin-glucan composition was established, were examined under the electron microscope as well as with phase fluorescence. The results indicate that the chitin-glucan complex shows a fibrillar arrangement and is localized in the so-called encircling region of the bud scar which is electron-transparent in ultrathin sections.

Folia microbiologica 18: in press.

3) Some Morphological and Physiological Properties of *Candida utilis* Growing Hypertrophically in Excess of Substrate in Two-stage Continuous Cultivation.

D. Vraná.

Abstract: In two-stage continuous cultivation with inflow of the fresh medium into the second stage, where $D_1 = 0.35\ h^{-1}$ and $D_2 = 0.45$, 0.60, 0.75, 0.90 and $1.1\ h^{-1}$, the growth rate, u_2, the division rate, $u\ d_2$, the size of cells and specific rate of RNA and DNA synthesis, K_{RNA} and K_{DNA}, were followed in the second stage. It was observed that the increasing dilution rate in the second stage has a significant influence on the physiological state of yeasts. Employing the dilution rate of 0.45, 0.60, 0.75 h^{-1} respectively, the doubling time of biomass formation and the cell generation time equaled and the curves of the specific rate of RNA and DNA formation had an identical course. At $D_2 = 0.9$ and $1.1.\ h^{-1}$ the curve K_{RNA} went up sharply, while the curve K_{DNA} rather slowly. The morphological consequence of the lagged rate of DNA synthesis is the formation of considerably enlarged cells or short filaments consisting of several cells or multiple budding mother cells. In such culture the biomass doubling time, u_2, reaches the values of "hypotrophic" growth and the value of the generation time, ud_2 markedly decreases. By polychrome differentiation of RNA and DNA using acridine orange in fluorescence microscope, no nucleic substance was found in daughter cells of multiple budding yeasts despite the fact that at least one daughter cell reached the size of its mother cell. Generally, it can be assumed that the maximum rate of DNA synthesis is reached at a lower D_2 than the maximum rate of RNA synthesis and that at a high D_2 the formation of DNA lags behind the formation of RNA. The discrepancy between the "inner capacity" of formation of both nucleic acids plays a decisive role in morphogenesis.

Advances in Microbial Engineering, Proc. 1st Internat. Symp., Mariánské Lázně, Czechoslovakia, August 28-Sept. 1, 1972. Eds. B. Sikyta, A. Novák, M. Novák, Interscience Publs., New York (in press).

4) Production of Native Protein from Yeasts.

Z. Fencl, F. Machek, K. Beran, V. Šillinger and J. Kejmar.

Our previous paper on mechanical disintegration of microorganism was used as a basis for preparation of protein from yeasts. The purpose of this study was to prepare a native tasteless protein applicable to the food industry. This protein can be given a meat texture. Isolated protein has a low content of nucleic acids. Conditions of protein release, reduction of nucleic acid content and isolation of protein from solution were studied. On the basis of the laboratory results, a semi-pilot continuous production line with production capacity of 1/2 kg of protein per hour was designed.

Advances in Microbial Engineering, Proc. 1st Internat. Symp., Mariánské Lázně, Czechoslovakia, August 28-Sept. 1, 1972. Eds. B. Sikyta, A. Prokop, M. Novák, Interscience Publs., New York (in press).

5) Automated Sampling During Continuous Cultivation.

V. Hromádka, K. Beran, J. Řičica, Z. Holan.

Folia microbiologica 17:513, 1972.

XXIV. Rutgers University, The State University of New Jersey, Institute of Microbiology, New Brunswick, New Jersey 08903, U.S.A. Communicated by J. O. Lampen.

I am spending the period from January 10 to about September 1, 1973 at the National Institute for Medical Research in Mill Hill, England, carrying on some

studies on penicillinase secretion by Bacillus licheniformis and working on a review on enzyme secretion by microorganisms. During my absence, Dr. S.-C. Kuo is in general charge of our work on yeasts. Dr. Paloma Liras from the University of Salamanca, Spain, has been examining the membrane and metabolic alterations produced by polyene antibiotics, and Dr. Aida Goldstein is working on improved preparations of the external glycoprotein and the internal carbohydrate-free forms of invertase. Dr. William J. Colonna completed his Ph.D. in 1972. His thesis was: The Chemistry and Structure of the Mannan from Saccharomyces mutant strain FH4C.

Recent papers and reviews from the laboratory are listed below.

1) General reviews of our research program were presented at the Yeast Symposium in Kyoto in March 1972:
Lampen, J. O., S.-C. Kuo, F. R. Cano and J. S. Tkacz. "Structural features in synthesis of external enzymes by yeast." 4th International Fermentation Symposium, Kyoto, Japan, March 19-25, 1973.

2) A general review was presented at the Yeast Protoplast Symposium in Salamanca, Spain:
Lampen, J. O., S.-C. Kuo and F. R. Cano. "Control of synthesis and secretion of exoenzymes." III International Yeast Symposium, Salamanca, Spain, October 2-5, 1973.

3) Dr. Kuo is presenting a paper entitled The Action of Antibiotics on Enzyme Secretion in Yeasts at the New York Academy of Sciences Symposium on "Mode of Action of Antibiotics on Microbial Walls and Membranes," June 6-8, 1973. Abstract: Cytochalasin A (CA), but not Cytochalasin B is a potent inhibitor of growth and invertase formation and secretion of Saccharomyces 1016. CA (10-20 μg/ml) inhibited uptake and accumulation of glucose, glucosamine and 2-deoxyglucose but only after 10-20 min incubation, and did not affect the transport of L-sorbose. With yeast extract-peptone grown cells, the uptake and incorporation of amino acids or uridine was highly sensitive to CA. In the presence of glucose (5 mM), uptake or incorporation of these precursors was greatly enhanced and became relatively insensitive to CA. Accumulation of alpha-aminoisobutyric acid (taken up by an energy-requiring process) was inhibited by CA in the absence but not in the presence of glucose. No leakage of UV-absorbing materials or K^+ or efflux of preloaded ^{14}C-alpha-aminoisobutyric acid was observed. The intracellular level of ATP fell rapidly in the treated cells. The findings suggest that CA does not directly block the transport of the various substrates, but rather inhibits in some manner an energy-generating process essential for their active uptake and accumulation.

4) Kuo, S.-C. and J. O. Lampen. Inhibition by 2-deoxy-D-glucose of synthesis of glycoprotein enzymes by protoplasts of Saccharomyces: Relation to inhibition of sugar uptake and metabolism. J. Bacteriol. 111:419-429, 1972.

5) Tkacz, J. S. and J. O. Lampen. Surface distribution of invertase on growing Saccharomyces cells. J. Bacteriol. 113:1073-1075, 1973.

6) Tkacz, J. S. and J. O. Lampen. Stereoregularity of chemically synthesized (1→6) alpha-D-mannopyranan as revealed by enzymic degradation with Arthrobacter alpha-D-mannanase. Carbohydrate Research 21:465-472, 1972.

7) Sentandreu, R. and J. O. Lampen. Biosynthesis of mannan in Saccharomyces cerevisiae. Isolation of a lipid intermediate and its identification as a mannosyl-1-phosphoryl polyprenol. FEBS Letters 27:331, 1972.

8) Sentandreu, R., M. V. Elorza and J. O. Lampen. Metabolic pathways of

Saccharomyces cerevisiae LK2G12 wall. Effect of cycloheximide. In Molecular Mechanisms of Antibiotic Action on Protein Synthesis and Membranes. Ed. by E. Muñoz, F. García-Ferrandez and D. Vasquez. Elsevier Publishing Co., Amsterdam, 1972, pp. 438-454.

9) Montenecourt, B. S., S.-C. Kuo and J. O. Lampen. Saccharomyces mutants with invertase formation resistant to repression by hexoses. J. Bacteriol. 114:233, 1973.

10) Kuo, S.-C., F. R. Cano and J. O. Lampen. Lomofungin - an inhibitor of RNA synthesis in yeast protoplasts: Its effect on enzyme formation. Antimicrobial Agents and Chemotherapy. In press, 1973.

11) Cano, F. R., S.-C. Kuo and J. O. Lampen. Lomofungin, an inhibitor of DNA-dependent RNA polymerases. Antimicrobial Agents and Chemotherapy. In press, 1973.

XXV. Leningrad Chemical-Pharmaceutical Institute, 14 Professor Popov Street, Leningrad 197022, U.S.S.R. Communicated by N. P. Elinov.

I) Post-graduate student A. K. Matveyeva (Department of Microbiology, Chief Dr. N. P. Elinov) recently completed her dissertation and supported the thesis in September 1972 about Structure and Characteristics of the Extracellular Polysaccharide Production by Pullularia (Aureobasidium) pullulans strain N 8.

Summary:

1. Composition and structure of production and laboratory samples of pullulan have been studied.
2. Pullulan is a branched glucan with a main chain of β-(C1→C3) connected glucopyranose units and each 3d unit has a side branch in position C6. The latter consists of α-(C1→C4) connected units of D-glucose.

```
   [      —α—(4G1—α—4G1)n1       ]
   [                 |           ]
   [                 | α         ]
   [                 |           ]
   [                 6           ]
 ——[—β—3G1—β—3G1—β—3G1—β—        ]n
```

The production sample had longer side branches than laboratory samples.

3. This pullulan is different from pullulan studied by Bender et al. (1959), Wallenfels et al. (1961, 1965) and others.
4. The general structure of pullulan is constant. It has a unit chain length equal to 3. Number of C1→C4 linkages fluctuated between 6 and 21%, C1→C3 linkages - 44-56% for 22 laboratory specimens.
5. Some derivatives of pullulan have been produced by chemical modification of its structure (carboxymethylpullulan, cyanethylic ether, derivative with epichlohydrin and others).
6. Some specimens of pullulan are antiviral substances (against influenza virus A_0/PR_8 and A_2/Hong Kong).

II) Change in employment.

Dr. N. P. Elinov was appointed as Rector (President) of Chemical-Pharmaceutical Institute of Leningrad since the 18th September 1972. At the same time he is chief, Department of Microbiology in the same Institute. His address is:

14, Professor Popov Street
Leningrad 197022, U.S.S.R.

XXVI. Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A OR6, Canada. Communicated by Byron F. Johnson.

1. The following article has been published recently.

Role of Mitochondria in the Sex-Directed Flocculation of a Fission Yeast.
G. B. Calleja.
Archives of Biochemistry and Biophysics 154, 382-386, 1973.

Cultures of Schizosaccharomyces pombe NCYC 132, a homothallic haplont, were grown anaerobically to stationary phase and then aerated. Cells flocculated within 1 hr of aeration. Competence for flocculation induction decayed as the cultures were allowed to age in stationary phase. Heat-killed cells were not inducible, but flocculated if induced before they were killed. However, massive ultraviolet irradiation, which resulted in the inability of the cells to form colonies when plated, did not stop induction. Added at the time of aeration, cyanide, azide, and dinitrophenol inhibited induction. So did membrane-specific drugs, such as nystatin and polymyxin, as well as inhibitors of protein synthesis such as cycloheximide, puromycin, and neomycin. Chloramphenicol, at saturating concentration, had no effect on flocculation induction when added at the time of aeration. But added to aerobically growing cells long before the last generation, chloramphenicol completely inhibited floc formation. The results indicate that induction of competence to form flocs requires mitochondrial function and cytoplasmic protein synthesis.

2. The following two articles are in press.

Ultrastructural Changes of the Fission Yeast (Schizosaccharomyces pombe) during Ascospore Formation. Archiv für Mikrobiol. (in press).
B. Y. Yoo, G. B. Calleja and G. F. Johnson.
Abstract: The fission yeast, Schizosaccharomyces pombe, a homothallic haploid strain NCYC 132, was induced to flocculate and conjugate and then ascosporogenesis was studied. It was found that two nuclei are formed during meiosis I and these nuclei divide again during meiosis II. The forespore membrane emerges at the beginning of meiosis II and elongates without fragmentation to enclose the nucleus and other cytoplasmic organelles, including mitochondria and ER. Meiosis occurs without fragmentation of the nuclear membrane. Immediately after the enclosure of the nucleus, the forespore membrane is resolved into two separate membranes. The inner one appears to become the spore plasmalemma and the outer one, if it persists, becomes a limiting membrane of the spore wall. In some cases, the outer membrane is seen to be ruptured. The site of spore wall materials is the space between the inner and outer membranes.

Cell Division in Yeasts. Movement of Organelles Associated with Cell Plate Growth of Schizosaccharomyces pombe. J. Bacteriol. (in press).
Byron F. Johnson, B. Y. Yoo and G. B. Calleja.
Abstract; Electron microscopy of dividing fission yeast cells show establishment of an annular rudiment (ar) of electron transparent material under the old cell wall as the first sign of elaboration of the cell plate. The ar grows centripetally, finally closing at the mid-point of the cell. During the inward growth of the ar it is thickened by addition of denser material which becomes the scar plug after fission; the electron transparent material is lost at fission. Lying always between the cytoplasmic membrane and the cell wall is a dark layer of variable thickness. This layer becomes markedly thickened into a fillet at the base of the centripetally growing cell plate. The fission process begins after the cell plate is completely elaborated. One striking feature of fission is the migration of dense material from the fillet at the base of the cell plate outwardly

through the matrix of the cell wall to its final resting place as a dark ring, a "fuscannel", adjacent to the fission scar. The inclusion of Golgi bodies in many sections suggests their involvement in cell plate elaboration, presumably through production of the dense bodies which are seen to fuse with the dark layer proximal to the growing cell plate.

XXVII. U.E.R. de Luminy, Département de Biologie, Laboratoire de Structure et Fonction des Biomembranes E.R., 143 CNRS - 70, route Léon Lachamp, 13288 Marseille, France. Communicated by E. Azoulay.

1. Distribution des enzymes et des cytochromes de *Candida tropicalis* cultivé sur alcanes.
M. Gallo, B. Roche, L. Aubert and E. Azoulay.
Biochimie 55, 195-203, 1973.
Summary: Intracellular localization of some enzymes and cytochromes of *Candida tropicalis* grown on alkanes was studied by fractionation of spheroplast lysates.

The adaptation to hydrocarbons causes a considerable increase in cellular levels of catalase and isocitrate lyase; the latter enzyme unlike that of *Saccharomyces cerevisiae* is recovered in both mitochondria and cytoplasm, under these conditions. The localization and levels of other activities of the Krebs and glyoxylate cycle are not modified.

Moreover, the growth on alkanes induces several microsomal enzymes (hydroxylase, alcohol and aldehyde dehydrogenases) capable of converting alkanes to their corresponding fatty acids, increases the level of a b-type microsomal cytochrome, and induces the formation of one or more soluble heme-like substances the nature and physiological role of which have not yet been determined.

2. Alkane oxidation in *Candida tropicalis*.
M. Gallo, J. C. Bertrand, B. Roche and E. Azoulay.
Biochim. Biophys. Acta 296, 624-638, 1973.
Summary: Reexamination of NAD^+ reduction by cell-free extracts of *Candida tropicalis* in the presence of n-decane provides conclusive evidence that this reaction is due to the contamination of some decane samples by an impurity, and does not correspond to a hydrogenation of the alkane itself. The actual pathway of alkane oxidation by *C. tropicalis* is a microsomal and inducible hydroxylase system. This system involving cytochrome P 450 and specific for NADPH is similar to the mammalian hydroxylase system.

The mitochondrial fragments of *C. tropicalis* contain an ATP-dependent transhydrogenase, the presence of which explains the fact that crude particle preparations, but not purified microsomes, can substitute NADH or NAD^+ for NADPH in alkane hydroxylation. The same transhydrogenase is also responsible for the previously reported observation that ATP increases NAD^+ reduction by these enzymic preparations in the presence of impure decane.

In the active microsomal particles, the hydroxylase system is associated with alcohol and aldehyde dehydrogenases, which are also present in mitochondria and which are partly solubilized in the course of some procedures of cell extraction. The induction of these dehydrogenases and of the hydroxylast system has been studied.

XXVIII. Department of the Army, U. S. Army Foreign Science and Technology Center, 220 Seventh Street NE, Charlottesville, Virginia 22901, U.S.A. Communicated by Edward Spoerl.

The following paper has recently been published:

Spoerl, E., J. P. Williams and S. H. Benedict. Increased Rates of Sugar Transport in *Saccharomyces cerevisiae* A Result of Sugar Metabolism. Biochim. et

Biophys. Acta 298:956-966, 1973.

Preincubation of yeast cells with glucose or other metabolic energy sources increased the rate of sorbose efflux 2- to 3-fold. Stimulated rates persisted for several hours, decreasing slowly. They were approximately halved by including K_m concentrations of highly competitive sugars such as deoxyglucose, glucose, fructose and mannose in sorbose efflux suspensions, and were greatly slowed at reduced temperatures. Inhibitors of energy metabolism blocked the rate stimulation, as did cycloheximide; added nitrogen sources increased the rate additionally. The rate of sorbose uptake was also increased, whereas that of dimethylsulfoxide, which enters the cell by simple diffusion, was not changed. Transport of arabinose and fucose also occurred at increased rates. The data indicate a change in the sorbose transport system rather than in membrane permeability. The change, apparently the synthesis of a transport system component, requires metabolic energy and involves protein synthesis.

XXIX. Research Laboratories, State Alcohol Monopoly, Alko, Box 350, 00101 Helsinki 10, Finland. Communicated by H. Suomalainen.

Heat Formation During Anaerobic and Aerobic Growth of Baker's Yeast.
E. Oura.
Proceedings of the 1st National Meeting on Biophysics and Biotechnology in Finland, ed. by L. Patomäki and A. Kiuru, Helsinki, January 1973, pp. 142-144.

During anaerobic growth the ΔH for energy-yielding catabolism of glucose is calculated to be -197 kcal/100g yeast d.m. formed (Y_{Gluc} = 15). During aerobic growth the ΔH is -271 kcal/100g yeast (Y_{Gluc} = 90). The values given in the literature and the author's results indicate the ΔH for anaerobic growth to be about -170 kcal and for aerobic conditions, of the order of -300 kcal/100g yeast formed. The close correspondence of these two sets of values indicates that the ATP formed in catabolism does not participate significantly in the generation of binding energy during cell material synthesis, but is dissipated so that almost all is released as heat.

Respiratory Enzymes in Baker's Yeast in Oxygen and Glucose Limited Continuous Cultivations.
E. Oura.
Abstract of paper presented at the Special Meeting of the Federation of European Biochemical Societies, Dublin, Ireland, April 1973.

A growth series was performed with yeasts using continuous cultivations and varying the intensity of aeration. The intensity was changed by varying the oxygen content of the gas mixture used for aeration. The experimental conditions were such that effects of catabolite repression on the formation of enzymes were eliminated. The activities of glycolytic and oxidative enzymes of yeasts grown under different aerations were investigated and distinct changes were noted. The cytochromes reached a maximum with only 5% oxygen in the aeration mixture; the first maximum for malate dehydrogenase could be seen at an even lower aeration level. The ability of yeast to respire glucose was most vigorous with 10% oxygen. The two oxido-reductases investigated also reached a maximum in this area. The growth was not, however, completely aerobic until also the enzymes of the glyoxylate cycle were completely developed. The increase in the activity of oxidative enzymes corresponds to the decrease in activity of many glycolytic enzymes. The results show that baker's yeast has a distinct and strong bias to transfer to an aerobic metabolism under oxygen-limited growth conditions where catabolite repression does not act. Yeast has even a superfluous potential to undergo oxidative metabolism under aeration conditions where the type of metabolism is determined by the limited oxygen supply.

The following publication has appeared since the last communication. Abstract of the report has been given in the Yeast News Letter in January 1973.

J. C. Londesborough and T. Nurminen. A manganase-dependent adenyl cyclase in baker's yeast, Saccharomyces cerevisiae. Acta Chem. Scand. 26:3396-3398, 1972.

XXX. Louisiana State University, Department of Food Science, Baton Rouge, Louisiana 70803, U.S.A. Communicated by S. P. Meyers.

The following has just been published:

1. The Microbial Degradation of Oil Pollutants. D. G. Ahearn and S. P. Meyers (eds.). Center for Wetland Resources, Louisiana State University Publication Number LSU-SG-73-01. 1973, 322 pp. Direct inquiries to: Publications Office, Center for Wetland Resources, Louisiana State University, Baton Route, LA 70803.
(or requests can be made directly to Dr. S. P. Meyers or to Dr. D. G. Ahearn, Department of Biology, Georgia State University, 33 Gilmer SE, Atlanta, Georgia 30303). Portions of the publication deal with activities of yeasts and moulds in degradation of oil pollutants.

2. From the Aquatic Microbiology Newsletter. April 1973. As a direct outgrowth of the Workshop on Microbial Degradation of Oil Pollutants held in Atlanta, Georgia, December 1972 (see December Newsletter), a separate Newsletter will be issued entitled OIL BIODEGRADATION NEWSLETTER. (Dr. D. G. Ahearn of Georgia State University, Atlanta, will be Co-Editor). This will be mailed only to those who work on oil pollution problems and on biodegradation of oil and petroleum products. Interested qualified researchers are invited to write to Dr. S. P. Meyers for information.

XXXI. Bulgarian Academy of Sciences, Institute of Industrial Microbiology, Sofia, Bulgaria. Communicated by V. Kostov.

Study of the Effect of Some Factors on the Preservation of Paraffin-Oxidizing Properties of Strains of the Genus Candida.
Il. Pashev, V. Kostov and V. Abadjieff.

Summary: The effect of some solid nutrient media was studied on the preservation of paraffin-oxidizing properties of 20 strains of yeast of the genus Candida. The studies were carried out in the course of almost two years.

Four kinds of solid nutrient media were used: 1) malt agar; 2) Pashev agar; 3) synthetic medium supplemented with 10 per cent of kerosene and 4) synthetic medium supplemented with peptone and 10 per cent of kerosene.

Two kinds of kerosene; Rumanian (Ploesti) and a mixture of Dolni Dabnik and Romashkinski (USSR) added respectively to the liquid nutrient media in the form of 10 per cent emulsion.

The results revealed that the strains depending on their capacity of assimilating n-alkanes from both kinds of kerosene can be divided into two basic groups: C. lipolytica (oxidative type) assimilating paraffins of both kinds of kerosene, and C. tropicalis (fermentative type) assimilating only paraffins of the mixed kerosene. This selective behaviour may be attributed, on the one hand, to the presence of paraffin fractions with a definite length of the carbon chain displaying different degree of assimilation rate, and on the other hand, to a possible presence of inhibiting substances.

It was found that cultures maintained on solid nutrient media with the addition of kerosene preserve their paraffin-oxidizing properties as good as in the case of their maintenance on media without any addition of kerosene.

(Publ. in: Bulletin of the Institute of Microbiology, Bulgarian Academy of Sciences, 23, 7-16, 1972).

XXXII. Miller Brewing Company, Milwaukee, Wisconsin 53201, U.S.A. Communicated by J. T. Rice.

Abstract of a paper presented at the convention of the American Society of Brewing Chemists, in New Orleans, May 1973.

The Kinetics of Diacetyl Formation and Assimilation during Fermentation. James F. Rice and J. Raymond Helbert.

The formation and the assimilation of diacetyl during fermentation is a result of the normal metabolism of yeast growth. It is useful for the present discussion to divide the fermentation period into a diacetyl formation phase (Phase-1) and an assimilation phase (Phase-II), their juncture being the point at which diacetyl concentration is maximal. Fermentations were run with the temperatures of the two Phases both the same (isothermal) and different (heterothermal). All fermentations were agitated under controlled conditions and utilized wort of fixed composition.

Under these conditions, both the formation and the assimilation of diacetyl follow the kinetics of a first order reaction, i.e., the diacetyl concentration changes exponentially with fermentation time. The diacetyl formation rate constant and the maximum diacetyl concentration are both primarily related to the amount of yeast growth, which in turn is related to the Phase-I temperature. The diacetyl assimilation rate constant is related to both the Phase-II temperature and the amount of yeast growth. Because both rate constants are temperature-related. Arrhenius plots can be constructed.

In isothermal fermentations with other conditions constant, the time for diacetyl formation, the time for diacetyl assimilation, and the time for maximum yeast growth all fit Arrhenius-type plots, over the temperature range examined (8.5°-22.2°C). Thus, the time to reach various points in a fermentation, including the end of fermentation, can be predicted. An equation is presented which permits the total fermentation time to be calculated for both isothermal and heterothermal fermentations.

A mechanism for diacetyl formation and assimilation is proposed in which the rate of yeast growth controls the rate of the biosynthesis of the precursor of diacetyl, viz., alpha-acetolactate. The production of this intermediate metabolite is ultimately controlled by the availability of fermentable carbohydrates.

XXXIII. Mikrobiologisches Institut, Swiss Federal Institut of Technology, Weinbergstrasse 38, Zürich, Switzerland. Communicated by A. Fiechter.

1. Recent publications:

H. Hug: Die Bedeutung der Lipide beim mikrobiellen Kohlenwasserstoffabbau. Thesis No. 5068.

A. Einsele: Der Einfluss von Rühren und Mischen in Reaktoren auf mikrobiologische Fliesskulturen. Chem. Rundschau No. 16, 3, 1973.

A. Einsele and A. Fiechter: Mischen und Rühren in der Fermentationsindustrie. Chemie-Anlagen und Verfahren 4, 57-58, 1973.

J.-R. Mor, A. Zimmerli and A. Fiechter: Automatic determination of glucose, ethanol, amino nitrogen and ammonia. Cell Counting. Data Processing. Anal. Biochem. 52, 614, 1973.

2. In press:

H. W. Blanch and A. Einsele: The kinetics of yeast growth on pure hydrocarbons. (Accepted for publication in Biotechnol. Bioeng.).

K. E. Weibel, J. R. Mor and A. Fiechter: Rapid sampling of yeast cells and automated assays of adenylate, citrate, pyruvate and glucose-6-phosphate pools. (Accepted for publication in Anal. Biochem.).

U. Flury and A. Fiechter: Purification and properties of malate dehydro-

genase from Saccharomyces pombe. (Submitted for publication to Eur. J. Biochem.).

XXXIV. Research Institute of Brewing (Jozo Shikenjo), 2-6 Takinogawa Kita-ku, Tokyo, Japan. Communicated by Yataro Nunokawa.

I would like to communicate some new knowledge obtained on non-foaming sake yeast.

Ouchi, K. and Nunokawa, Y. Non-foaming mutants of sake yeast: Their physico-chemical characteristics. J. Ferment. Technol. 51, 85-95, 1973.
Abstract: Some physico-chemical properties were compared between parent (wild type) cells and non-foaming mutant cells of a sake yeast, Kyokai No. 7, to attempt to discover differences in their cell surface structures on which the froth head forming ability might depend. While the parent cells were almost completely removed into the froth fraction by a froth flotation, only about 14% of the cells of the non-foaming mutant BU9-5 were removed under similar conditions. When the parent cells were shake in water-benzene mixtures, they accumulated in the solvent phase. They also moved into the solvent phase in a series of water-n-paraffin and water-n-alcohol systems; in the former, the cells transferred into the solvent having a number of carbon atoms greater than n-hexane and, in the latter, they transferred into the solvent with a number of carbon atoms greater than those in n-heptanol. Mutant cells, on the other hand, did not transfer so well into any of the solvents tested. The parent cells aggregated upon addition of sucrose monopalmitate in concentrations of 500-1,000 μg/ml, while the mutant cells did not. In alcohol solutions, both the parent and mutant cells aggregated at concentrations above a certain critical level, but the concentration was a little higher for the parent than for BU9-5. Electrophoretic mobility curves showed that the parent cells had isoelectric points near pH 3.5 but the cells of BU9-5 had no isoelectric point and were negative in charge at any of the pH values tested. The parent cell aggregated with cells of Lactobacillus plantarum at pH 2-5, while BU9-5 cells did not aggregate at any of the pH values tested. Acid titration curves of the intact cells were almost the same in the parent as the mutant cells. Both types of cells were also similarly agglutinated by concanavalin A. From these results, differences in the cell surface structure between the parent and the mutant are discussed.

XXXV. Research Institute for Viticulture and Enology, 886 15 Bratislava, Matuškova 21, Czechoslovakia. Communicated by Dr. E. Minárik.

The following manuscripts have been recently accepted for publication:

1. E. Minárik: Occurrence of very rare yeast species on grapes and in musts (in German) - Die Wein-Wissenschaft, Wiesbaden, GFR 1973.

   Kluyveromyces veronae, Hansenula subpelliculosa, Kloeckera corticis and Trigonopsis variabilis have been isolated and identified on grape berries and in must. Morphological and physiological characters of these unfrequent yeast species are discussed briefly and compared with the description in Lodder et al. (1970).

2. E. Minárik: Reduction of sulphate and sulphite by wine yeasts (in Slovak) - Kvasný průmysl (Prague 1973).

   The ability of increased sulphate uptake and sulphite formation by several species of the genus Saccharomyces could be confirmed repeatedly in laboratory and small-scale cellar tests. Sulphite formation during fermentation may be considerably increased by elevated initial sulphate concentrations and (or) pasteurization or sterilization of the must prior to fermenta-

tion, but only in strains with explicit sulphate uptake and sulphite forming ability. Culture conditions (temperature, pH, inoculum etc.) do not significantly influence sulphite formation. The importance of the application of pure yeast starters with little or no SO_2 and H_2S forming capability is considered.

3. E. Minárik: Sulphate reduction and sulphite formation, its taxonomic value in yeast classification (in French) - Progress in Viticultural and Enological Research, Vol. VII, Bratislava 1974.

   Taxonomic aspects of sulphate reduction and sulphite formation, respectively, in several Saccharomyces species and strains are discussed. The importance of the selection of yeast strains with minimal sulphite or sulphide formation for winemaking is underlined.

XXXVI. Massachusetts Institute of Technology, Cambridge, Mass. 02139, U.S.A. Communicated by D. M. Levine.

Methanol Utilization by a Thermotolerant Strain of Hansenula polymorpha.
David W. Levine.
Submitted to the Department of Nutrition and Food Science on January 24, 1973, in partial fulfillment of the requirements for the degree of Master of Science.

A yeast capable of growth on methanol as its carbon-energy source at temperatures greater than 35°C was isolated from soil samples and identified as a strain of Hansenula polymorpha. A continuous enrichment culture with mineral salts-methanol medium, at 37°C was used to select for this organism. The isolate designated DL-1 grows optimally with a maximum specific growth rate of 0.22 $hours^{-1}$ at pH 4.5-5.5, and at temperatures of 37-42°C in mineral salts-methanol medium supplemented with biotin and thiamine-HCl. Growth was observed in a chemostat at temperatures up to 50°C with strong growth at 45°C. The growth yield of DL-1 on methanol is 0.36 gram dry cell weight/gram methanol and the yield on oxygen is 0.37 gram dry cell weight/gram O_2. At methanol concentrations of less than 0.5% (v/v), DL-1 exhibits no inhibition of growth by methanol. The Monod growth model was found to describe the dependence of growth on methanol concentration very well, with a value for the Monod constant, K_s, equal to 120 mg methanol/liter. At greater than 1% methanol, growth rate decreases with increasing methanol.

Protein content of DL-1 is 46% and total nucleic acid content varies from 5.0% to 7.0% with increasing growth rate from 0.08 to 0.20 $hours^{-1}$. The amino acid profile of our yeast protein indicates that it could serve as a good source of food protein. Feeding studies with rats show no toxic effects.

Thesis Supervisor: Dr. Charles L. Cooney.
Title: Assistant Professor of Biochemical Engineering

XXXVII. Horticultural Products Laboratory, Horticultural Research Institute of Ontario, Vineland Station, Ontario LOR 2EO, Canada. Communicated by R. V. Chudyk.

Yeast Research at the Horticultural Research Institute of Ontario.
R. V. Chudyk, A. M. Adams and Pamela M. Gadd.

1. Introduction. The Horticultural Research Institute of Ontario is responsible for the integration of all horticultural research in Ontario funded by the Ontario Ministry of Agriculture and Food. The institute consists of several sections one of which is the Horticultural Products Laboratory (HPL). The HPL section itself consists of a wine unit, a food processing unit, a biochemistry unit, a physiology unit and a microbiology unit. The HPL section is situated in the Niagara Peninsula of Ontario where over 90% of Canada's grapes and wines are produced. The microbiology unit maintains an intimate dialogue with individual wineries, but also conducts micro-

biological research prompted by the Canadian Wine Institute. This report outlines the scope of the yeast research effort in the Microbiology Unit of the HPL.

2. Culture Collection. Our laboratory maintains a collection of over 250 industrial yeasts. The collection is a research collection as well as a depository for the commercial wine industry of Ontario. Occasionally, the yeast collection is the subject of specific investigations. Several years ago, our laboratory studied factors affecting sulphide production by wine yeasts. The effects of various fungicides on wine yeasts have also been investigated. A long-term project on the yeast collection has been concerned with culture preservation methods. Preservation methods investigated have included slant culture, freeze-drying and more recently, liquid nitrogen storage. Each year our laboratory and the wine industry cooperate in the production of frozen yeast starters. The wineries produce bulk starters of their wine yeasts. Our laboratory freezes the starter cultures, then distributes them back to industry as required.

   Our laboratory is continually searching for, and along with industry, evaluating new wine yeasts isolated from our 35 acre experimental vineyard containing 15,000 vines representing 300 varieties. One wine-yeast search method involves a spontaneous or natural fermentation of various musts. The Wine Unit at HPL produces over 100 varietal wines yearly. The Microbiology Unit ferments small volumes of sulphured and unsulphured musts. The dominant yeasts from each fermentation are evaluated as potential wine yeasts.

3. Isolation Media for Airborne Winery Yeasts. Twenty-one different commercial media were evaluated for ability to yield the maximum airborne winery yeast count. Duncan's Multiple Range Test indicated that Yeast Morphology Agar was far superior than 20 other media including Malt Extract Agar, Wort Agar, W.L. Medium and Sabouraud Medium. A manuscript for publication has been prepared.

4. Microflora of Ontario Wineries. Yeast Morphology Agar was used to survey the yeasts and bacteria in Ontario wineries. Areas sampled included bottling rooms, storage cellars and laboratories. Results indicated that the numbers and type of yeasts and bacteria varied between wineries and between various locations in the same winery. A manuscript for publication has been prepared.

5. Microbiology of Bottled Wines. A major study is currently underway for developing improved media and techniques for detecting viable yeasts in bottled wines. Over twenty-five media are being evaluated for ability to detect yeasts in wine. Two-hundred domestic and imported wine brands will be tested. Results on one-hundred wine brands examined to date indicate that traditional media (i.e. Wort Agar and W.L. Medium) used by the wineries (and recommended by membrane filter manufacturers) are not reliable indicators of the viable microbial count in bottled wines. Several manuscripts have been prepared for publication.

6. Yeast Populations in Vineyards. The Microbiology Unit is interested in the *in vivo* life cycle of yeasts in our experimental vineyard. We have examined the vineyard soil, air plus various parts of the grape vine for several seasons. We have isolated many yeasts from almost every vineyard source. One of our more interesting observations is the fact that we have microscopically observed yeast colonies on the bud. The colonies, when observed, tend to be only one cell thick. We have also observed "sexual-like" stages on the grape bud in the form of 2-spored asci. No other ascus types have been observed and attempts to germinate have been unsuccessful. The yeast life cycle literature tends to describe the *Saccharomyces* life cycle *in vitro*. Our laboratory would be interested in establishing a dialogue with any laboratory which has observed *Saccharomyces* asci *in vivo*.

7. HPL Wine Workshop. The Horticultural Products Laboratory conducted a wine workshop (March 7 and 8, 1972) for the Ontario wine industry. This workshop was not intended as a formal series of lectures but an informal seminar for the technical winery staff. The Microbiology Unit led a seminar and the following yeast topics were discussed: Methods for detecting winery microflora; yeast classification; yeast preservation; microscopic observation of wine yeasts; and biological (yeast) stabilization of wines.

XXXVIII. Brief News Items

1. Dr. J. Lodder has moved from Delft. Her new address is: Jerfaasplantsoen 9, Bennekom, The Netherlands.

2. I am looking forward to working in Professor Wiken's laboratory, Delft, Holland, during part or, hopefully, all of the forthcoming academic year. My research will relate primarily to evaluation of a yeast fermentation system for detection of both tumor and antitumor chemicals. Visits from other researchers shall be most welcome.

John G. Kleyn
University of Puget Sound
Tacoma, Washington 98416

3. The following publication will appear in a forthcoming issue of Antonie van Leeuwenhoek:
"Electron microscopy of septa in ascomycetous yeasts" by N. J. W. Kreger-van Rij and M. Veenhuis.

N. J. W. Kreger-van Rij
Oostersingel 59
Groningen, Holland

4. The following progress has been made in organizing the yeast groups here in Japan.

A small conference of yeast researchers - 2 or 3 representatives from each of the 8 or 9 groups - was held in Kyoto on May 1. A brief summary of the conclusions of our meeting follows:

1) A joint symposium on Yeast Biology should be held each year for the benefit of the various yeast groups. The first of these is tentatively scheduled for the end of this year, in Tokyo.
2) Dr. Nagai will act as the Japanese editor for Yeast News Letter and will also handle appropriate related business matters.
3) A simple mechanism for the exchange of information between the yeast groups has been worked out.

Yasuji Oshima
Department of Fermentation Technology
Faculty of Engineering
Osaka University
Yamada-Kami, Suita-Shi
Osaka, Japan 565

5. Research news at the Mycology Laboratory, Plant Protection Institute, Agricultural Research Service, U.S.D.A., Beltsville, Maryland 20705.

Paper presented: Taxonomy and Systematics of the Hemiascomycetes (Hemiascomycetidae), L. R. Batra, at the International Symposium on Taxonomy of Fungi, 1973, Univ. Madras, Madras, Madras 5, India.

A key to the genera of the Hemiascomycetes and a list of accepted, doubtful, or rejected genera were included in

the presentation. Also included in the paper was a discussion of comparative morphology and life history of selected Hemiascomycetes. The proceedings of the symposium will be published in the near future.

Current Research: Role of yeasts in fermentation of plant products of India. Isolation and identification of yeasts present, and their role of transformation of the plant products.

L. R. Batra, Research Mycologist

6. Experimental Inhibition of Cell Wall Formation and of Reversion in *Nadsonia elongata* and *Schizosaccharomyces pombe* Protoplasts.

Marie Havelková
Department of General Biology, Faculty of Medicine, Purkyně University, Brno, Czechoslovakia.

Protoplasma 75, 405-419, 1972.

Summary: The growth, cell wall regeneration, and the reversion of the protoplasts of *Nadsonia elongata* and *Schizosaccharomyces pombe* cultivated in nutrient media containing snail enzyme was studied by light and electron microscopy. The protoplasts grew in the presence of snail enzyme and an incomplete cell wall composed of fibrils was formed on their surface. Thus, the presence of snail enzyme inhibited the completion of cell wall structure and, consequently, the reversion of the protoplasts to normal cells. The transfer of these protoplasts to medium free from snail enzyme led first to the completion of the cell wall and then to the reversion of the protoplasts to normal cells. The reported experiments confirmed that the regeneration of the complete cell wall preceded the protoplast reversion.

7. Cell Wall Growth During the Cell Cycle of *Schizosaccharomyces pombe*.

Eva Streiblová and A. Wolf
Department of General Microbiology and Laboratory for Optical Microscopy, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia.

Zeitschrift für Mikrobiologie 12:673-684, 1972.

Summary: Cell wall growth of an asynchronous culture of *Schizosaccharomyces pombe* was studied by fluorescence microscopy and time-lapse cinematography. The cell wall growth of individual cells is a time-ordered process which can be divided into 4 stages: post-division stage, stage of growth initiation, stage of extension growth, and constant length stage. Different aspects of these stages are discussed. During the exponential phase of growth the population has a rather stable structure from the point of view of division scar numbers. The significance of the division scars for the study of cell wall growth and multiplication is discussed.