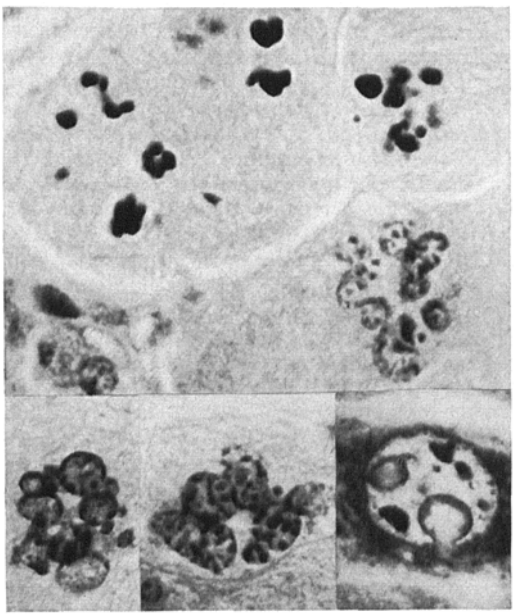
Einheitskern aufgenommen. Bisweilen kommunizieren sie mit dem übrigen Zytoplasma, sei es unmittelbar (Figur), sei es durch einen schmalen Verbindungsgang, der dann später noch «obliterieren» oder völlig verschwinden kann. Die colchicinbedingten nuclearen Plasmainklusionen sind also keine Bildung des Ruhekernes, sondern ein postmitotisch entstandenes Phänomen. Dasselbe gilt für viele andere membranumzogene («indirekte») Kerneinschlüsse, insbesondere in menschlichen und tierischen Tumorzellen.



Postmitotische Entstehung cytoplasmatischer Kerneinschlüsse (rechts unten) bei der Verschmelzung von Chromosomenbläschen. Leberzellen Maus. 24 h nach der letzten von 4 Colchicininjektionen.

In den Plasmainklusionen ist oft Glykogen und Fett vorhanden. Mitochondrien sind dagegen, obwohl elektronenoptisch nachgewiesen[4], lichtoptisch nur selten darstellbar. Sicher sind sie von den aufquellenden Chromosomenbläschen grossenteils beiseite gedrängt worden. Doch können sie, eingeschlossen, auch bis zur Unkenntlichkeit verändert werden, da die Stoffwechselverhältnisse in den vom übrigen Zelleib mehr oder weniger abgeschnürten Arealen unzureichend sind. Aus dem gleichen Grunde wird das gesamte eingelagerte Plasma häufig sekundär verändert und, ganz oder teilweise, entweder hyalinisiert oder verflüssigt. Ebenso kann die zunächst stets vorhandene Membran zugrunde gehen[14–17]. Darnach liegt die denaturierte Plasmaportion unmittelbar im Karyoplasma; sie ist damit sekundär zu einem «freien» oder «direkten» Kerneinschluss geworden.

Summary. The colchicin-induced nuclear inclusions are a postmitotic phenomena. Fusing chromosome vesicles often include a portion of cytoplasm, surrounded by persisting parts of the vesicle membranes ('indirect' or 'membraned' n.i.). This membrane sometimes degenerates; the n.i. then lies free in the karyoplasm (secondarily 'direct' or 'free' n.i.).

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[14] F. EHRENBRAND und E. LINDNER, Ref. Dtsch. Ges. Elektronenmikrosk. *9*, 12 (1959).
[15] E. H. LEDUC und J. W. WILSON, J. Biophys. Biochem. Cytol. *6*, 427 (1959).
[16] H.-W. ALTMANN, Virchows Arch. *334*, 236 (1961).
[17] W. THOENES, J. Ultrastruct. Res. im Druck (1964).

A Method for the Selection of Auxotrophic Mutants of the Yeast *Schizosaccharomyces pombe*

The methods reported[1,2] for the concentration of auxotrophic mutants in yeast are only of limited value for the isolation of mutants occurring at a frequency of 10^{-8} to 10^{-6}.

In order to develop an efficient concentration technique, a method analogous to the penicillin method for bacteria[3] was tried. 2-Desoxyglucose, glucosamine and fluoride were used to block cell wall biosynthesis by inhibiting the enzyme phosphoglucomutase. With this method it was possible to enrich auxotrophic mutants in reconstruction experiments. Because of the high rate of mutation to resistance to these inhibitors, experiments along this line were not reproducible and were discontinued.

The method finally adopted for the concentration of spontaneous mutants is based on the inositol requirement of the wild type of *Schizosaccharomyces pombe* and is fashioned after the method of LESTER and GROSS[4] for the isolation of auxotrophic mutants in Neurospora. This method involves inositol-less death of growing, prototrophic cells and the preservation of non-growing mutant cells on inositol-less minimal medium.

Inositol-less plates are prepared by pouring 30 ml of minimal agar medium[5], lacking inositol, in standard petri dishes which contain a sterile, analytical grade filter paper disc. The filter paper serves only a mechanical purpose: it prevents the cracking of the agar when the plates are supplemented with inositol and growth factors.

Wild-type cells are grown in liquid minimal medium[5] supplemented with L-malate at a concentration of 0.01 m to a density of 10^8 cells/ml, centrifuged and resuspended

[1] A. G. MOAT, N. PETERS JR., and A. M. SRB, J. Bacteriol. *77*, 673 (1959).
[2] D. PITTMAN, E. SHULT, A. ROSHANMANESH, and C. C. LINDEGREN, Can. J. Microbiol. *9*, 103 (1963).
[3] L. GORINI and H. KAUFMAN, Science *131*, 604 (1960).
[4] H. E. LESTER and S. R. GROSS, Science *129*, 572 (1959).
[5] R. MEGNET, Arch. Jul. Klaus-Stiftung *33*, 299 (1959).

in saline. The cell concentration is adjusted to 10^8 cells/ml and 0.1 ml of this suspension is spread on the surface of well dried inositol-less plates and incubated at 30°C.

After incubation for 5 to 10 days, the agar is lifted at the edge with a spatula and 1 ml of a solution with 1 mg of inositol/ml and growth factors, at suitable concentrations, is pipetted onto the bottom of the plate. The agar is then gently dropped back and the plates are incubated for 4 days at 30°C. Replica plating[6] on minimal or other selective media reveals the auxotrophic mutants.

This method can also be used for the concentration of UV-induced mutants. UV-treated cells are plated after pre-incubation in liquid medium which allows phenotypic expression.

With this method a number of mutants were isolated which could not be recovered by standard procedures. These mutants include amino acid auxotrophs, a fair number of adenin specific and pyrimidine requiring mutants.

Recently it was possible to concentrate mutants which are unable to use glucose, mannose, maltase, fructose and sucrose as carbon sources. These mutants can grow on gluconate or glycerol and are probably hexokinase deficient. Mutants requiring gluconate for growth could also be isolated.

Zusammenfassung. Spontane oder mit UV induzierte auxotrophe Mutanten der Hefe *Schizosaccharomyces pombe* können auf einem inositol-freien Medium, auf welchem prototrophe Zellen, aber nicht die Mutantenzellen absterben, selektioniert werden. Mit der beschriebenen Methode ist es auch möglich Kohlehydratmutanten zu isolieren.

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[6] J. LEDERBERG and E. M. LEDERBERG, J. Bacteriol. *63*, 399 (1952).

A New Antifungal Antibiotic Produced by *Streptomyces sp.* Ac_2435

The polyene group of antifungal antibiotics are widely distributed in soil Actinomycetes. Several surveys have been made[1-4], and it has been fairly established that they have in common high activity against yeasts and a variety of fungi including plant and human pathogens. The authors have been able to isolate a strain of *Streptomyces sp.* (designated as Ac_2435) which showed high activity against fungal test organisms.

The active substance was produced in synthetic Czapek-Dox liquid medium modified by replacing $NaNO_3$ with $(NH_4)_2SO_4$. The substance was produced in stationary flask cultures at 28–30°C and the broth was harvested on the 6th to the 8th day of incubation. The chemical purification of the antibiotic was done by extracting the broth with *n*-butyl alcohol and further purified by concentration and repeated precipitation with ether. The substance was finally obtained as an amorphous brown powder by chilling at 4°C followed by drying *in vacuo* at low temperature. The anti-microbial spectrum of the purified substance was determined by the cup method of assay, and its minimum inhibitory concentration is shown in Table I.

Table I. Antimicrobial spectrum of Ac_2435

Test fungus	Minimum inhibitory concentration (μg/ml)
Saprophytic	
Saccharomyces cerevisae	6.0
Saccharomyces ellipsoidus	6.5
Torula sp.	5.0
Penicillium notatum	8.2
Penicillium chrysogenum	7.0
Syncephalastrum sp.	9.0
Cunninghamella sp.	8.0
Trichoderma viridae	10.4
Plant pathogens	
Aspergillus niger	10.0
Aspergillus oryzae	30.5
Alternaria solani	16.0
Curvularia lunata	4.5
Fusarium oxysporum	20.0
Helminthosporium sativum	8.0
Glomerella cingulata	8.0
Human pathogens	
Candida albicans	18.5
Candida tropicalis	17.0
Trichosporon cutaneum	15.0
Sporotrichum schenecki	20.2
Microsporum gypseum	12.0
Microsporum audouini	10.5
Microsporum canis	9.0
Trichophyton rubrum	10.0
Trichophyton sulfurcum	11.2
Trichophyton interdigitale	10.0
Epidermophyton floccosum	15.4

The substance is readily soluble in *n*-butyl alcohol, methanol, pyridine and formamide. It is moderately soluble in water and acetone and insoluble in benzene ether, petrol ether, chloroform and carbon tetrachloride. The antibiotic is sensitive to light and temperatures above 40°C. Complete inactivation was noticed at 100°C and after an exposure of the butanolic solution for 1 h to strong sunlight. Its activity could, however, be retained by storing the substance dry at low temperature (below 10°C) in the dark for several months.

The m.p. of the substance was 160°C (decomposition) and optical rotation D $27.8 = +40$ (C 2% methanol). Elemental composition of the substance was determined as follows: C 43.01%, H 7.68% and O 49.31%. It was found to be homogeneous as evidenced by paper chro-

[1] S. BALL et al., J. gen. Microbiol. *17*, 96 (1957).
[2] J. D. DUTCHER et al., in *Therapy of Fungus Diseases* (Little, Brown and Co., Toronto 1955), p. 168.
[3] R. A. PLEDGER and H. LECHEVALIER, Antibiot. Ann. (1955–56), p. 249.
[4] Z. VANEK et al., J. gen. Microbiol. *18*, 649 (1958).