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).

Table II. Comparative *in vitro* activity of the antibiotic produced by Ac_2435 with some of the known tetraene antibiotics

Test organism	Minimum inhibitory concentration (μg/ml) of				
	$Ac_2(435)$[a]	Nystatin	Pimaricin	Rimocidin sulphate	Amphotericin A
1. *Candida albicans*	18.5	35.0	34.0	18.0	16.0
2. *Candida tropicalis*	17.0	42.2	40.0	16.0	17.5
3. *Saccharomyces cerevisae*	6.0	18.5	16.0	15.0	6.0
4. *Saccharomyces ellipsoidus*	6.5	18.0	20.0	8.0	15.0
5. *Torula sp.*	5.0	18.0	12.0	12.0	13.4
6. *Aspergillus niger*	10.0	45.0	17.0	25.0	10.0
7. *Aspergillus oryzae*	30.5	100.0	100.0	100.0	18.0
8. *Alternaria solani*	16.0	35.0	30.2	26.0	12.0
9. *Curvularia lunata*	4.5	8.0	10.0	15.0	4.0
10. *Fusarium oxysporum*	20.0	100.0	35.0	20.0	32.0
11. *Glomerella cingulata*	8.0	10.0	4.0	25.0	4.5

[a] Purified antibiotic for comparison.

matographic and counter current distribution studies. The UV absorption spectrum had a maxima at 288, 305 and 320 mμ, indicating the presence of a conjugated *tetraene* structure. Comparative assays with known tetraene antibiotics have been carried out against yeast and several other fungi, and the results are given in Table II. The toxicity test of the antibiotic was carried out on mice in which S.C. LD_{50} was 400 mg/kg of the body weight.

Zusammenfassung. Ein neues antifungales Antibioticum, *Streptomyces sp.* $Ac_2(435)$ wurde mit breitem Spektrum isoliert. Die aktive Substanz ist ein amorphes, nicht-kristallines weisses Pulver, das in Wasser mässig löslich ist. Die Substanz ist lichtempfindlich und bei 100°C inaktiv. Vergleichsproben mit bekannten Substanzen ergaben eine Tetraenverbindung.

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Department of Microbiology, Bose Institute, Calcutta (India), December 3, 1963.

Ciliated Smooth Muscle Cells in the Uterus of the Rat

In the course of a study on the action of steroid hormones upon the fine structure of the uterine smooth muscle cell of the spayed rat, a relatively large number of cilia was observed. Since, to our knowledge, only one report exists[1] upon the occurrence of cilia in smooth muscle cells, and the significance of their appearance in such an apparently abnormal site is at present only speculative, a brief description of them in the smooth muscle cells of an organ in which they have not been noticed before seemed warranted.

The observations were performed on the uteri of 30 castrated adult albino rats which were routinely fixed in 1% Osmium tetroxide buffered at pH 7.4 and embedded in Araldite. Thin sections obtained with an LKB Ultrotome were stained with potassium permanganate and observed with a Philips 100 B electron microscope.

The general appearance of the cilia is similar to that previously described in the smooth muscle cells of other organs[1]. Generally, only one cilium could be observed in a single cell, but occasionally two cilia located close to each other could be seen. Most of the cilia appeared close to the nucleus, deeply embedded in the cell, but at times they were close to the cell surface, with most of the shaft in the intercellular space.

In our observations the cilia assumed different appearances. Generally, they were observed as being composed of a basal body from which a short shaft buds into a small vesicle. The shaft measured at least 200 mμ in width and its length was variable according to the orientation of the section. The largest one found measured 2.5 μ. In transverse sections the cilia appeared to be composed of 9 double fibres arrayed peripherally around an area of very low electron density. In no case was evidence of central fibrils found. The structure of the basal body resembled that of the centriole, and the peripheral fibres of the cilia appeared to be continuous with those of the basal body. No basal plate could be seen between the ciliary shaft and the basal body. Most of the cilia were surrounded by a clear space of variable width limited by a membrane which in favourable sections appeared continuous with the plasma membrane. In this case the plasma membrane invaginated until the level of the junction between basal body and cilium. At this place the clear space is wider than the remainder which surrounds the shaft. Generally, the cilia appeared close to a centriole.

In the ultra-thin sections and small cellular areas observed with the electron microscope, a quantitative estimation of the abundance of cilia in the uterine smooth

[1] S. SOROKIN, J. Cell Biol. *15*, 363 (1962).