Kølmark, H. G. Ureaseless mutants in Neurospora crassa. The presence of the enzyme urease in Neurospora hos been known for many years (Srb ond Horowitz 1944 J. Biol. Chem. 154: 129). Mutation to on ureaseless condition is therefore to be expected.

In the present experiments it was first found that urease could very easily be demonstrated directly on a growing colony, simply by rooking a small piece of pH-indicator paper (range about pH 6-B) in on 8% solution of urea in water and placing it in contact with the mycelium or conidia. A color change towards the alkaline takes place in a few minutes due to liberation of ammonia when urea is enzymatically hydrolysed.

This change in pH was utilized in the screening methods finally worked out for isolation of ureaseless mutants.

Strains of Neurospora: Some earlier experiments with macroconidiating strains of colonial morphology did not succeed in the isolation of ureaseless mutants. A microconidiating, small colonial strain, 398-28 A, was then tried in the expectation that mutants might more easily become phenotypically expressed when induced in mononucleate conidia. A morphological mutant, m-25, derived from this strain after treatment with ultraviolet light, was used in some of the later experiments. m-25 forms very small and extremely dense colonies.

Experimental Procedures: Suspensions of conidio in saline were irradiated with UV and, after appropriate dilutions, incubated in 25 ml of liquid minimal medium in petri dishes kept at 21-22°C. Under these conditions the colonies grow below the surface ond adhere to the gloss bottom of the plate. After 6-7 days they ore ready to be tested for urease activity.

The medium is then decanted off. With careful handling the colonies stick to their growth place on the gloss. They ore rinsed twice with saline. After the last rinsing the plates or. placed in a slanted position for a few minutes to let superficial saline drain off. A filter paper is moistened with a mixture of 4% (or stronger) urea and a pH indicator (brom cresol purple or brom thymol blue) in the range 5.5-7.5, adjusted to the lower pH with a weak phosphate buffer (ionic strength 0.01). When the paper is pressed against the colonies with a gloss plate, all normal colonies change the indicator color in a few minutes. Non-reacting colonies ore isolated into tubes with minimal medium. Established cultures are retested later in comparison with the original strain.

In on alternative procedure, th. UV-treated conidia or. spread on the surface of solid medium. A whit. net of nylon fabric, ca. 3 threads per mm, is placed on top of the inoculated medium. The colonies, appearing between the meshes, ore after 3 days overlayered with 15 ml of liquid minimal medium ond th. incubation continued for 5 days more. The liquid medium is added because of the finding that the enzymatic reaction proceeds more readily when the mycelium has grown submerged. The net is stripped off with port of the colonies attached, when ready for testing. The screening test is don. on this replica as described above. Isolations or. mod. from the untested part of the colonies left on the plate.

Two cultures out of a total of 58 isolator were by retesting found to be entirely lacking in urease activity, as evidenced by no shift towards alkalinity during 4-5 days when a small amount of mycelium was incubated at 34°C in 0.25 ml of 4% urea, dissolved in 0.01 ionic strength phosphate buffer. The reaction is started at clear yellow (pH ca. 6) with the indicator brom thymol blue. Th. original strain gives a strongly positive reaction in 2 hours.

Several other isolates appear to have a decreased urease activity. The frequency of ureaseless mutants was found to be about 1 per 10^4 survivors. The ureaseless mutants or. unable to grow on urea as the sol. source of nitrogen, while the original strains grow well. Otherwise, the mutants show no decreased vitality with regard to germination of conidio or ascospores nor in mycelial growth under the conditions observed.

Genetic testing: On. of th. ureaseless mutants, u-9 A, was crossed to th. wild typ strain 74-ORB-la. The ureaseless character was found to segregate regularly at 1st or 2nd reduction division in dissected asci. Ascospore cultures with urease-less recombined into wild type morphology or. fully fertile also as protoperithecial parents. Genetic testings to identify the linkage group are in progress, but so for it con only be stated that it seems not to be situated in the linkage groups 1, II or VII. It 'is proposed that the mutant phenotype described above be designated by the symbol ur, since this symbol seems not previously to be in use as a locus symbol in Neurospora.

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