This mutation is probably of a genetic nature, and may be associated with sex, as has been shown by the writers[4,5] in another fungus. The *M* type is physiologically as well as morphologically distinct from the *C* type, and where mass transfers of inoculum are employed the *M* type is apt to become predominant. The appearance of the *M* type *de novo* in a *C* type culture is a function of physiological age. Pure cultures of the *C* type which are maintained in a state of youth by frequent transfer (always made by means of conidia) tend to remain free of the *M* type.

Presence of the *M* type in what appears to be a normal culture often remains undetected unless the culture be frequently analyzed by the single spore technique,[2] when some of the single spores will be found to produce the normal sporulating *C* type and others to produce the non-sporulating *M* type with which is associated an increased production of yellow pigment. This *M* type (non-sporulating and pigment-producing) is presumably the form which various workers have reported as being a poor producer of penicillin. The presence of the *M* type in cultures used for inoculum would be expected therefore to result in decreased penicillin production.

If our interpretation is correct, it would appear that the highest yield of penicillin probably could be obtained by frequently making single spore cultures of the fungus and choosing the most productive of these for large-scale operations, whether these be slight variations within the *C* type or even in the *M* type.

If it is desired merely to return the present stock culture to its highest sporulating condition it is suggested that a procedure somewhat as follows be adopted. Gently flush conidia from the agar slant stock culture with sterile water. Flood the surface of an agar medium in a plate or flask with this conidial suspension; pour off the excess suspension and incubate the inoculated medium in diffuse light. Harvest the new crop of conidia soon after they are formed, again using the flushing method to avoid carrying over mycelial fragments into the suspension. This spore suspension from this fresh culture should be pure for the *C* type. Cultures must never be scraped to obtain inoculum if the *M* type is to be avoided.

M types rarely mutate. If an *M* type is found which produces a satisfactory yield of penicillin it probably may be propagated by mass transfer without recourse to the above methods. If it is desired to distribute this inoculum through a liquid to serve in place of a spore suspension the mycelial colony may be cut up by means of a Waring blender.[6]

It should be clear that to maintain penicillin production at maximum levels the highest yielding clone of *P. notatum* should be used and that this clone be kept monotypically pure and free from recurring mutants. It should be evident also that where biological assay of penicillin is practiced, the assay organism too must be perpetuated in a monotypically pure state. Only when this is done is it possible to effectively standardize the processes of penicillin production and assay.

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[4] H. N. Hansen and W. C. Snyder, *Phytopath.*, 30: 787, 1940.

[5] *Idem, Amer. Jour. Bot.*, 30: 419, 1943.

[6] C. F. Andrus, *Phytopath.*, 31: 566, 1941.

AN ANTIBIOTIC SUBSTANCE FROM SPECIES OF GYMNOASCUS AND PENICILLIUM

THE antibiotic substances from three different fungi, namely, *Aspergillus clavatus, Penicillium patulum* and *Penicillium claviforme,* and variously called clavacin, claviformin and patulin have proved to be identical.[1] Raistrick *et al.*[1] have elucidated the chemistry as anhydro-3-hydroxymethylene-tetrahydro-γ-pyrone-2-carboxylic acid (patulin). This substance has now been isolated from two more mold species. The first was an undefined species of *Gymnoascus,* labelled 5070.1, and kindly furnished by Dr. Thom. The second was *Penicillium* sp. freshly isolated from soil in the course of a survey. Cultivated for 7 to 10 days on Czapek-Dox medium containing 3 per cent. corn steep liquor, the *Gymnoascus* filtrate inhibited *Escherichia coli* at 1/100 and *Staphylococcus aureus* at 1/50. The active agent was adsorbed on 1 per cent. norite, eluted with acetone, and the eluate concentrated *in vacuo* to a thin syrup. After standing overnight in a refrigerator, crystals appeared. They were separated by filtration and recrystallized twice from hot 50 per cent. ethanol. The white crystals melted at 109°. A mixed melting point with crystalline clavacin (m. 109.5°), from *Aspergillus clavatus* kindly supplied by Dr. Waksman, showed no depression. The substance analyzed as follows: C, 54.72; H, 3.98 (theoretical: C, 54.53; H, 3.93); molecular weight (cryoscopic in ethylene dibromide), 195. $C_7H_6O_4$ requires 154. The 2,4-dinitrophenylhydrazone derivative began to darken at 190° and did so progressively up to 250° C. without melting. The

[1] I. R. Hooper, H. W. Anderson, P. Skell and H. E. Carter, SCIENCE, 99: 16, 1944; S. A. Waksman, E. S. Horning and E. L. Spencer, SCIENCE, 96: 202–3, 1942; H. Raistrick, J. H. Birkinshaw, S. E. Micheal and A. Bracken, *Lancet,* 245: 625–34, 1943; B. P. Weisner, *Nature,* 149: 356–7, 1942; F. Bergel, A. L. Morrison, A. R. Moss, R. Klein, J. Rinderknecht and J. L. Ward, *Nature,* 152: 750, 1943; E. Chain, H. W. Florey, M. A. Jennings and D. Callow, *Brit. Jour. Exptl. Path.*, 23: 202–5, 1942.

acetyl derivative melted at 116° (Raistrick *et al.* give 116–118°; Hooper *et al.*, 116–117°). The substance is neutral, is rapidly destroyed by alkali and decolorizes permanganate. These properties correspond with published data on patulin and clavacin, and this substance is, therefore, identical with the other two.

The pure material is quite toxic for mice. L.D. 100 = 12.5 mg per 20 g mouse. It failed to protect mice against lethal *Salmonella schotmulleri* infections in the highest doses tolerated.

A crystalline substance isolated as above with slight modifications from culture filtrates of *Penicillium* sp. also proved to be identical with clavacin. The substance melted at 109.5° and showed no depression mixed with authentic clavacin. It analyzed as follows: C, 54.92; H, 4.04. Other properties coincide with those of clavacin.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

INOCULATION OF MEDIA FOR MOLD CULTURE

IN the cultivation of molds in large flasks or bottles it is sometimes difficult to obtain a uniform degree of inoculation and to produce an even growth over the entire surface of the medium. A technique used successfully in this laboratory with several species of Penicillium employs a suspension of spores in a medium containing gum tragacanth in which a small amount of lanolin has been emulsified. The particles of lanolin apparently assist in buoying the spores to the surface of the culture medium and holding them there until germinated.

A homogenous emulsion is prepared by warming and stirring 2.5 g gum tragacanth and 0.5 g lanolin in 100 ml of water. Thirty grams of the mixture is placed in a 125 ml Erlenmeyer flask, together with five 12–15 mm glass marbles, the flask is plugged with cotton and sterilized. The flask is then rotated or shaken to emulsify the lanolin while being cooled to 30° C or below. Flasks of gum-lanolin mixture prepared in this way may be stored in the refrigerator indefinitely. To use this gum-lanolin mixture to prepare a spore suspension, the contents of one flask, including the marbles, are poured onto a spore culture grown on agar in a 250 ml flask. The flask is now shaken gently for several minutes with a circular motion in a horizontal plane to cause the marbles to roll over the spore-bearing suface. The spore suspension is further diluted for use by adding 25 ml of sterile water. The resulting suspension measures 45–50 ml and in the case of *Penicillium notatum* suffices to inoculate 15 or more 3-liter Fernbach flasks, the area of the medium in each being about 270 square centimeters. The inoculated flasks are thoroughly agitated by shaking just before incubation and are then allowed to remain undisturbed. This method is readily adapted to a sixfold increase in scale by growing the sporulation culture in a 3-liter Fernbach flask and modifying the rest of the procedure accordingly. For each Fernbach flask use 180 g of tragacanth-lanolin emulsion, increase the number of marbles to about a dozen and finally dilute with 150 ml of sterile water.

Methyl cellulose in place of gum tragacanth was not satisfactory because it did not properly emulsify the lanolin. Cetyl alcohol in place of lanolin or an eightfold increase in the amount of lanolin inhibited mold growth. If the medium being inoculated contains much suspended matter which settles out, the inoculation is less satisfactory. Presumably the material settling to the bottom counteracts the buoyant effect of the lanolin particles. This difficulty is corrected by filtration of the medium. The incorporation of a wetting agent, such as 0.1 per cent. Ivory soap or 0.4 per cent. Aerosol A.Y. in the gum-lanolin emulsion facilitated the loosening of spores from the mycelium but inhibited spore germination and mold growth.

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