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Nitrification by Soil Fungi

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(Received November 16, 1959)

From the time of WINogradsky's researches about the end of the last century, the process of nitrification has been considered to be brought about solely by the classical autotrophic nitrifying bacteria. Although there have been isolated reports in the past on nitrite formation by heterotrophic microorganisms, chiefly bacteria, the consensus of opinion among microbiologists was that such nitrification did not occur. There were valid reasons for this disbelief, inasmuch as the amounts of nitrite reported were extremely low and could in most cases have been due to several possible errors inherent in this type of work. Most of the earlier work which suggested or claimed nitrification by heterotrophic bacteria has been mentioned by FISHER et al. (1956).

In recent years, more evidence has been forthcoming to support the suggestion that heterotrophic nitrification may be more common than has so far been realized, though, quantitatively, this may still be less important than that of the classical nitrifiers. Thus, both bacteria (JENSEN 1951, HUTTON and ZOBELL 1953, FISHER et al. 1956) and Actinomycetes (ISENBERG et al. 1952) have been implicated in this process. That fungi can also bring about such transformation was first demonstrated by SCHMIDT (1954) whose results have been confirmed by us in related studies briefly reported elsewhere (IYENGAR and TRILOCHAN SINGH HORA 1959). It is our object here to provide additional data and examine the phenomenon of nitrification by soil fungi in greater detail.

Experimental Methods

Fungi were isolated from soil by the conventional dilution plate method on Czapek's solution agar or potato-dextrose agar containing fairly high concentrations of the antibiotics dihydrostreptomycin sulphate (250 µg/ml) and potassium penicillin G (300 units/ml) to inhibit all bacterial growth. Subsequent to isolation the organisms were maintained on potato dextrose agar.

All glassware used in the experiments was acid-cleaned and rinsed several times with glass-distilled water. Double-glass-distilled water and analytical grade reagent chemicals were used in the preparation of reagents and media.

pH measurements were made using the glass electrode.

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The same basal medium as was used by Schmidt (1954) was employed to screen the fungi for their nitrifying capacity, with one of the following nitrogen sources:

1. ammonium sulphate 0.07 percent; peptone 0.4 percent; sodium nitrite 0.05 percent (all w/v). The first two media were used for both nitrite and nitrate production, the last one only for nitrate production.

The media were used in amounts of 15 ml per 100 ml. Pyrex Erlenmeyer flask and autoclaved at 15 lb/sq. i. for 20 minutes.

For inoculation, spores were taken from heavily sporulating cultures a week to ten days old, using a moistened loop and taking care not to pick up either the underlying mycelium or agar. After dispersing the spores in water, uniform aliquots of 0.05 ml were aseptically transferred to the culture flasks. Incubation was at 22-25°C in a static condition and for varying periods as indicated in the text. At the end of the incubation period the mycelium was filtered off and the culture filtrate was used to test for both nitrite and nitrate. All tests were run in duplicate using two sets of controls: 1. Uninoculated medium and 2. medium inoculated with the fungus spores just prior to running the colour tests. All test filtrates were decolourized with charcoal before determining nitrite or nitrate.

Gries-Ilosvay reagent was used for the detection and estimation of nitrite in suitably diluted culture filtrates. The estimations were made with the aid of a Lumetron Photoelectric colorimeter, Model 402 E, and using the 515 M green filter. The phenoldisulphonic acid method was used to determine nitrate photometrically, with the 420 M blue filter. Where nitrite was present together with nitrate, total nitrate was determined after oxidizing the nitrite with a few drops of hydrogen peroxide.

Results

Out of the eighty cultures of soil fungi included in the preliminary screening trials several were capable of bringing about nitrification to varying degrees.

Examination of these cultures showed that they belonged mostly to either Aspergillus or Penicillium.

Nitrite and Nitrate Formation by Aspergilli

Among the aspergilli, two cultures were observed to produce both nitrite and nitrate in the medium containing peptone. Quantitative experiments were then run to assess the extent of nitrification brought about by these two aspergilli. Three authentic strains of Aspergillus flavus Link, obtained from the Indian Type Culture Collection, I.A.R.I., New Delhi, were also included as they were observed to produce both nitrite and nitrate in the same preliminary experiments.

The soil isolates of Aspergillus which were subsequently examined by standard methods (Thom and Rapo 1945) were also found to be Aspergillus flavus. The results are given in Table 1.

Among the nitrate- and nitrite-producing A. flavus strains, only the soil isolate F. 43 showed nitrate production in the sodium nitrite medium. The results of these experiments are indicated in Table 2. The growth of the organism on the nitrite medium was much less than usually observed.