INFLUENCE OF NUTRIENTS ON LYSIS OF FUNGAL HYPHAE IN SOIL

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Summary

Live fungal hyphae lyse faster in soil previously supplemented with glucose, peptone, or blood and bone meal than in non-supplemented soil. Whether or not the supplement increases the number of Actinomycetes or bacteria, either effect will result in more rapid lysis of hyphae. When peptone is added to soil which already contains fungal hyphae, Actinomycetes increase rapidly and the hyphae lyse. But with glucose as a supplement the fungus grows and coexists with the small increase in other microorganisms. After depletion of this supplement the starved hyphae are induced to lyse by the presence of other soil microorganisms.

I. Introduction

Most fungal hyphae when added to natural soil are rapidly lysed (Lockwood 1960; Bumbieris and Lloyd 1967). Their destruction is associated with the presence of other soil microorganisms, especially bacteria and Actinomycetes, because soil devoid of these microorganisms is non-mycolytic (Lockwood and Lingappa 1963; Lloyd and Lockwood 1966). Moreover, soil bacteria (Mitchell and Alexander 1963) and Actinomycetes (Lockwood 1959) will lyse living fungal mycelium in culture.

The purpose of this work was to observe the effect of added nutrients on the survival of fungal hyphae in soil, and on the growth of other soil microorganisms.

II. Materials and Methods

Fusarium solani f. *phaseoli* (Burkh.) Snyd. & Hans. and *Thielaviopsis basicola* (Berk. & Br.) Ferr. were used as the test fungi. Soil (Urrbrae loam) was obtained from a field under pasture for 5 years (Bumbieris and Lloyd 1967), and contained approximately $8 \cdot 0 \times 10^6$ bacteria and $3 \cdot 0 \times 10^6$ Actinomycetes per gram as determined by the dilution-plate method. Soil agar (Bunt and Rovira 1955) was used for estimating the bacterial populations, and chitin agar (Lingappa and Lockwood 1962) for Actinomycetes. After collection the soil was adjusted to 17–18% moisture content and stored in plastic bags at room temperature. The time in days for lysis of 90% of fungal hyphae was observed by a direct method (Lingappa and Lockwood 1963; Bumbieris and Lloyd 1967).

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Nutrients were added to soil by one of the following methods: (1) soil was mixed with 0·4% (w/w) glucose, peptone, or blood and bone meal 10 days before addition of fungal hyphae; (2) one drop of a 0·1% aqueous solution of glucose, ammonium nitrate, peptone, glucose–ammonium nitrate, or distilled water was added simultaneously with fungal hyphae to the surface of soil in a 5-cm Petri dish, and then the same nutrient again added on each of the following 3 days; (3) small disks (5 mm diameter) of either Czapek–Dox agar (with 0·05% yeast extract, but only 1% sucrose) or water agar, or sections 1 cm long of native oat grass (*Themeda avenacea*), all previously colonized by the test fungi, were placed on soil in 5-cm Petri dishes. In some cases the substrates were covered with 2–3 mm of loose soil.

III. Results

(a) *Effect of Added Nutrients on Lysis of Fungal Hyphae in Soil*

When fungal germlings were added to soil previously supplemented with glucose, peptone, or blood and bone meal, *F. solani* f. *phaseoli* hyphae lysed in 3–4 days, and hyphae of *T. basicola* in 5 days. In unsupplemented soil the same fungi lysed in 5 and 6 days respectively. The numbers of bacteria and Actinomycetes were $8 \cdot 4$ and $1 \cdot 5 \times 10^6$ respectively in unsupplemented soil. When soil was supplemented with glucose, peptone, or blood and bone meal the number of bacteria increased to $16 \cdot 9$, $19 \cdot 5$, and $18 \cdot 4 \times 10^6$ respectively. The actinomycete population remained constant with the glucose supplement, but increased to $14 \cdot 5$ and $7 \cdot 6 \times 10^6$ respectively with the peptone and blood and bone meal supplements.

However, when nutrients were added to soil simultaneously with fungal hyphae and on each of the following 3 days, then with peptone-supplemented soil the hyphae of *F. solani* f. *phaseoli* and *T. basicola* lysed in 4 and 5 days respectively; with ammonium nitrate, glucose–ammonium nitrate, or distilled water supplements in 5 and 6–7 days respectively; and with glucose supplement in 7 and 11 days respectively. Initially fungal hyphae grew in all supplemented soils, especially with glucose where growth was both abundant and sustained. Based on counts made of bacterial colonies and conidial chains of Actinomycetes recovered daily from soil in plastic peelings, bacteria increased slightly (from 2–5- to 6–10-fold) in soil supplemented with glucose or peptone, but did not increase in soil supplemented with ammonium nitrate or glucose–ammonium nitrate. On the other hand, Actinomycetes increased with all supplements (from 2–5- to 6–10-fold with glucose and ammonium nitrate supplements, and from 2–5- to 16–20-fold with glucose–ammonium nitrate), and especially with peptone where the increase was greater than 50-fold. Thus, supplementing soil with glucose stimulated fungal growth and delayed lysis, whereas peptone both stimulated growth of a large actinomycete population and promoted lysis of fungal hyphae.

(b) *Growth of Fungal Hyphae from a Colonized Nutrient Substrate*

When a nutrient substrate previously colonized by a fungus was added to soil in a Petri dish, hyphae grew along the soil surface. With Czapex–Dox agar as the substrate growth of *F. solani* f. *phaseoli* and *T. basicola* extended approximately 12 and 9 mm respectively in 4 days. During the same period growth from colonized

grass stems was 25 and 15 mm respectively, for the two test fungi, while with water agar as a substrate only a few hyphae of *F. solani* f. *phaseoli* grew, and these lysed within 4 days. By 8 days most hyphae growing from the different substrates had lysed.

When the above work was repeated, but this time burying the substrates in soil, results were similar, although it was not possible to measure the growth accurately. However, hyphae in soil lysed more slowly (about 10–12 days) than those on the soil surface.

IV. Discussion

Nutrients such as glucose, peptone, or blood and bone meal, when added to soil, stimulate growth of Actinomycetes and bacteria. After utilization of these nutrients and subsequent increase in microbial numbers, fungal hyphae then added to this soil lyse faster than in untreated soil. It does not matter whether the supplement increases the numbers of Actinomycetes or bacteria, for either effect will result in a more rapid lysis of hyphae, at least in moist soil such as was used in these experiments.

However, when a nutrient is added to soil which already contains fungal hyphae, survival of the hyphae will depend on their ability to utilize the supplement in competition with other soil microorganisms. With peptone as a supplement, actinomycete numbers increase rapidly and lysis of hyphae occurs. But with glucose the fungus grows and coexists for some time with other microorganisms. This suggests that, in our soil at least, fungi grow vegetatively provided that suitable nutrients are available (high carbon to nitrogen ratio), and the numbers of other microorganisms are not overwhelmingly high. Presumably a similar situation occurred when Cook and Snyder (1965) planted bean seed in soil inoculated with *F. solani* f. *phaseoli*. The germinating seed exuded sugars and amino acids which stimulated growth of adjacent microorganisms, resulting in rapid lysis of the fungal hyphae. But in the vicinity of the developing hypocotyl the exudate contained mainly sugars (high carbon to nitrogen ratio), and the hyphae grew and ultimately infected the bean plant.

Fungal hyphae can also grow into soil from a colonized organic substrate such as a piece of plant residue. While the fungus is able to translocate nutrients from the substrate to the growing tips, growth will continue outwards. But after depletion of nutrients the hyphae lyse, leaving behind any spores previously formed in the soil. Warcup (1957) also observed that the fungus, *Rhizopus arrhizus*, grew outwards from pieces of wheat stubble partly buried in soil. Presumably the nutrients in parts of soil devoid of pieces of organic matter are inadequate for sustained growth of the hyphae. The fungus becomes starved and, under the influence of other soil microorganisms, is induced to lyse.

V. References

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