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Nitrification by *Fusarhim* Species Grown under Oligotrophic Conditions

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Abstract: Laboratory study was conducted to determine the nitrification of ammonium sulphate, via nitrite, to nitrate by *Fusarium* species grown under oligotrophic conditions. The addition of ammonium sulphate led to a marked increase in the concentration of nitrate, with a slight increase in nitrite, in most species. The maximum amount of nitrate was recorded in a medium inoculated with fungi of *Fusarium solani* (3.4 µg/ml) and *Fusarium oxysporum* (2.3 µg/ml). These species exhibited the largest content of protein. *Fusarium ciliatum* (0.7 µg/ml) reached the lowest amount of protein production when grown under oligotrophic conditions.

Key words: Oligotrophic,nitrification, fungi, ammonium oxidation, nitrite, nitrate *Fusarium* spp.

Introduction

Microorganisms are generally grown in the laboratory in nutrient-rich medium containing large amounts of sugars, nitrogen, phosphorus and other nutrients which are considered essential for their growth, Natural environments, in contrast, contain only trace amounts of nutrients. Most soils can be regarded as being oligotrophic because they are generally considered to contain insufficient carbon to allow the continuous growth of fungi (Lynch, 1982; Fry, 1990).

While data on microbial growth on nutrient-rich media were being rapidly accumulated, a few early studies began to reveal that a number of bacteria, actinomycetes and fungi appeared to be able to grow on plain water agar or even in apparently nutrient-free, distilled water (Wainwright *et al*., 1991).

Microorganisms which are adapted for growth under these conditions are called oligotrophs (Wainwright, 1988). A wide range of fungi have been shown to be capable to grow cligotrophicaliy, such as species of *Aspergillus, Trichoderma*, *Fusarium* and Penicillium (Tribea and Mabadeje, 1972; Wainwright, 1987; Shimna *et al.,* 1989; Barakah, 1992). The ability of fungi to grow oligotrophicafly has a number of important biotechnologically, medical and environmental implications (Wainwright *et al*., 1991).

The present work was carried out with the aim of studying ammonium oxidation *in vitro* by *Fusarium* species grown under oligotrophic conditions.

Materials and Methods

Culture and growth medium: This research project was conducted *in vitro* in our Central Laboratory of Teachers College at Riyadh region of Saudi Arabia during a year of 2000. In this experiment *Fusarium solani* (Mart.) Saco., *Fusarium oxysporum* Schlecht., *Fusarium coeruleum* (*Lib*.) Sacc. and *Fusarium ciliatum* Link were grown on carbon and nitrogen free silica gel as described by Shimna *et al*. (1989). The basal medium used in this study was free of both nitrogen and carbon, consisting of : KH_2PO_4, 1 g/l; $MgSO_4.7H_2O$, 0.5 g/l; KCl, 0.5 g/l and $FeSO_4.7H_20$, 0.01 g/l in 1000 ml of pure distilled water, adjusted to pH 6.8 with 2 N NaOH, dispensed into Erlenmeyer flasks (100 ml in 250 ml flasks) and amended with 100 µg/ml of nitrogen as ammonium sulphate, sterilized by autoclaving at 120°C for 20 minutes. After cooling the flasks were then inoculated with a single disc (13 mm) cut from 14 days culture of *Fusarium* spp. grown on free carbon and nitrogen silica media following eight transfers. The inoculated flasks were then incubated in triplicate at 25°C on a reciprocal shaker (100 throws minute) for 4 weeks. A triplicate of the flasks were removed at 7 days intervals and contents filtered through Whatmen No.1 filter paper. Uninoculated controls were also included to account for any non-biological nitrification.

Determination of protein and nitrogen ions: The mycelium was collected after filtration and dispensed into a test tube. Protein content of *Fusarium* sp, grown oligotrophically in liquid medium was determined using the Folin phenol reagent (Herbert *et al*., 1971). Ammonium was determined according to the indophenol blue method (Wainwright and Pugh, 1973); nitrate using an Orange 1 method described by Middleton (1959) and nitrite colorirnetrically as described by Hesse (1971) pH of the medium was measured with a glass electrode.

Results

All *Fusarium*, species grown oligatrophically in liquid medium contained low content of protein (Fig 1.). *Fusarium solani* exhibited the highest content of protein which was 31 µg/100 ml of media at the end of the incubation period followed by *Fusarium oxysporum* with 26 µg/100 ml of media. On the other hand the fungus of *Fusarium ciliaturn* reached the lowest amount of protein with 12 µg/100 ml of media at the end of the incubation period.

Changes in pH of medium inoculated with *Fusarium* species grown under oligotrophic conditions are shown in Fig. *2.* The pH of the medium declined steadily in all treatments in the first week. The fungus *Fusarium solani* was recorded the highest reduction of medium pH that were 6.0 after 4 weeks of growth. In the case of *Fusarium oxysporum* and *Fusarium coeruleum* the final pH values of the media were 6.4 and 6.5 respectively. The final reading of media pH after *Fusarium ciliatum* growth showed no significant change and was probably due to the less growth observed with this fungus. *F. solani* produced maximum protein and lowered the medium PH.

Figure 3 shows ammonium assimilation by *Fusarium* species grown oligotrophicalfy in a liquid medium. *Fusarium solani* appears the most active in oxidizing ammonium, forming nitrate. The fungus *Fusariurn solani* was recorded the highest assimilation of ammonium that were 12 µg/ml. While the lowest assimilation of ammonium was observed in *Fusarium ciliatum* with 45 µg/ml at the end of the incubation period. All of *Fusarium* species tested oxidized ammonium leading to the formation of nitrate, via nitrite in a liquid medium under oligotrophic conditions (Fig. 4-7). This decline in

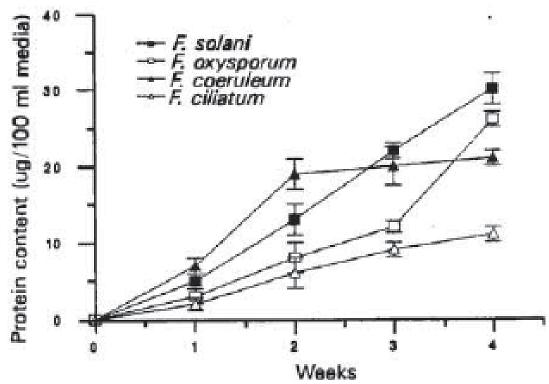
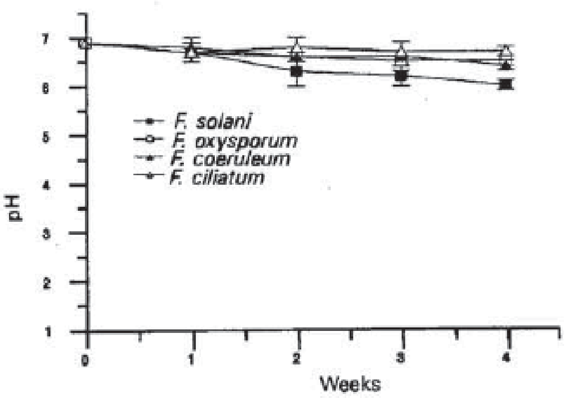


Fig. 1: Protein content of *Fuserium* species grown oligotrophically on liquid medium lacking carbon and nitrogen, all values are means of triplicates ± S.D



Fgt. 2: Changes in pH of oligatrophic liquid medium inoculated with *Fusarium* species, all values are means of triplicates ± S.D

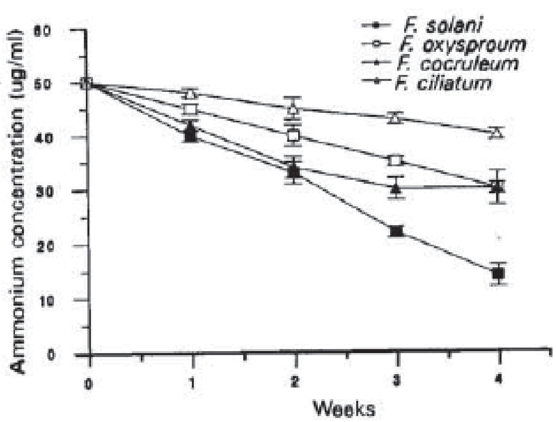


Fig. 3: Ammonium assimilation by *Fusarium* species grown oligotrophically in a liquid medium lacking carbon and nitrogen, all values are means of triplicates ± S.D

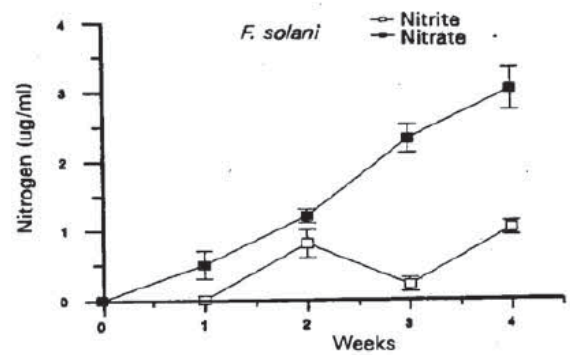


Fig. 4: Nitrification of ammonium (100 µg/ml) by *Fusarium solani* grown oligotrophically on liquid medium, all values are means of triplicates ± S.D

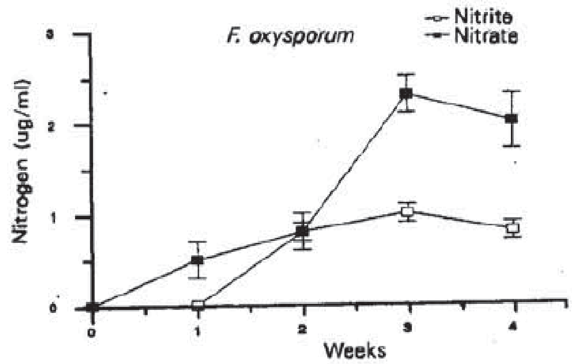


Fig. 5: Nitrification of ammonium (100 µg/ml) by *Fusarium oxysporum* grown oligotrophically on liquid medium, all values are means of triplicates ± S.D

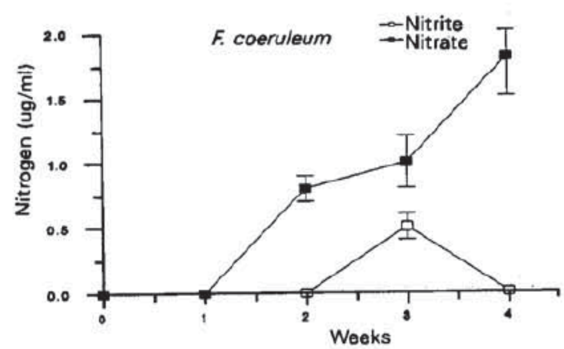


Fig. 6: Nitrification of ammonium (100 µg/ml) by *Fusarium coeruleum* grown oligotrophically on liquid medium, all values are means of triplicates ± S.D

ammonium was associated with a concomitant increased in net nitrite and nitrate production. The concentration of added ammonium decreased more rapidly in *F. solani* compared to the other *Fusarium* species tested. *F. solani* followed by *F. oxysporum* were recorded the highest nitrification of ammonium that were forming 3.4 and 2.3 µg/ml of nitrate respectively at the end of the incubation period (Fig. 4, 5). While the lowest nitrification of ammonium was observed in *F. ciliatum* with 0.7 µg/ml of nitrate at the end of the incubation period (Fig. 7). Therefore, *F. solani* and *F. oxysporom* were particularly active in ammonium nitrification process forming the maximum amount of nitrate at the end of the incubation period.

Actually, the concentration of nitrite ions never exceeded the

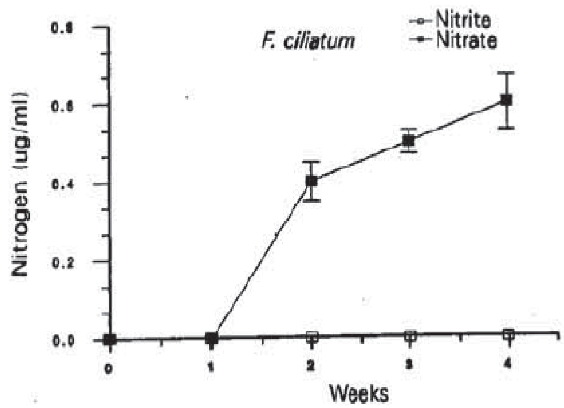


Fig. 7: Nitrification of ammonium (100 µg/ml) by *Fusarium ciliatum* grown oligotrophically on liquid medium, all values are means of triplicates ± S.D

concentration of nitrate formed by aft of the Fusarium species. The results show a small amount of nitrite in all cases with some exception, such that there was no amount of nitrite detected in the *F. coeruleum* and *F. ciliatum* (Fig. 6, 7). Nitrite ion is usually considered to be intermediates and rarely exceed the concentration of nitrate. As a result, nitrite ion was only formed transiently in trace amounts towards the end of the incubation period by *F. solani* and *F. oxysporum* (Fig. 4, 5).

Discussion

It is relatively difficult to study the protein content of oligotrophic growth because of the small amount of biomass produced under these condition (Wainwright *et al.,* 1991). However, as Fig. 1 shows. protein determination can be used as a reliable measure of fungal biomass. Thus, this parameter should be useful as a means of studying the small amounts of fungal biomass produced under low nutrient conditions, i.e. soil (Lynch, 1982; Fry, 1990). The amount of protein produced by individual fungi being seen to differ over an 4 weeks incubation period. Fungus of *F. solani* produced the largest protein content over the incubation period consistent with the findings of previous studies (Shimna *et al*., 1989; Barakah, 1992).

it was found after 4 weeks of incubation that the growth of *Fusarium* species in a liquid medium lacking carbon and nitrogen caused a marked decrease in media pH. The pH dropped after the fungi grew in the oligotrophic medium, the decrease in pH being broadly in proportion to the fungal growth (Herbert *et al*., 1971; Al-Falih and Wainwright, 1995). Results (Fig. 2) showed that the decreases in pH values were correlated with growth increase of *Fusarium* species in the liquid medium. The final reading of media pH after *Fusarium ciliatum* growth showed no significant change and was probably due to the less growth observed with this fungus. *F. solani* produced maximum protein and lowered the medium pH.

The oxidation of ammonium, as measured by both loss of ammonium and increases in nitrite and nitrate, varied from one fungus to another. Since the significant differences of protein production by *Fusarium* species, which are capable of grown under oligotrophic conditions, we were interested in determining their ability in this respect compared with each other. *Fusarium solani* appears the most active in oxidizing ammonium, forming nitrate. In the nitrification of ammonium all of the *Fusarium* species studied produced between 0.1 - 2.3 µg/ml nitrate over the 4 weeks incubation period, while *Fusarium solani* produced 3.4 µg/ml nitrate.

Since nitrite ion is usually considered to be intermediates and rarely exceed the concentration of nitrate. So the nitrite ion was only formed transiently in trace amounts towards the end of the incubation period by some of *the Fusarium* species similar results were reported earlier (Barakah, 1992; Al-Falih and Wainwright, 1995).

This study provide evidence of the nitrification of ammonium sulphate, via nitrite, to nitrate by *Fusarium* species grown under oligotrophic conditions. The addition of ammonium sulphate led to a marked increase in the concentration of nitrate, with a slight increase in nitrite, in most species.

These fungi are able to survive and grow in a medium free of carbon and supplemented only with ammonium sulphate as *a* sole nitrogen source. The maximum amount of nitrate was recorded in a medium inoculated with fungi of *Fusarium solani* and *Fusarium oxysporum.* These species exhibited the largest content of protein. While *Fusarium ciliatum* reached the lowest amount of protein production when grown under oligotrophic conditions.

References

Al-Falih, M.K. and M. Wainwright, 1995. Nitrification *in vitro* by a range of filamentous fungi and yeasts. Applied Microbiol. Lett., 21: 18-19.

Barakah, F.N.I., 1992. Observations on the oligotrophic growth of fungi. Ph.D. Thesis, University of Sheffield, UK.

Fry, J.C., 1990. Oligotrophs. In: Microbiology of Extreme Environments, Edwards, C.A. (Ed.). Open University Press, Milton Keynes, pp: 93-116.

Herbert, D., P.J. Phipps and R.E. Strange, 1971. Chemical Analysis of Microbial Cells. In: Methods in Microbiology, Norris, J.R. and D.W. Ribbons (Eds.). Vol. 5, Academic Press, London, New York, pp: 209-344.

Hesse, P.R., 1971. A Textbook of Soil Chemical Analysis. John Murray Publisher, London.

Lynch, J.M., 1982. Limits to microbial growth in soil. J. Gen. Micrbiol., 128: 405-410.

Middleton, K.R., 1959. The use of the orange I method for determining soil nitrates and a comparison with the phenol-sulphonic acid method for certain soils of Northern Nigeria. J. Sci Food Agric., 10: 218-224.

Shimna, M.P., M. Wainwright and K. Killham, 1989. Observations on oligotrophic growth of fungi on silica gel. Mycol. Res., 93: 529-534.

Tribea, H.T. and S.A. Mabadeje, 1972. Growth of moulds on media prepared without organic nutrients. Trans. Br. Mycol. Soc., 58: 127-137.

Wainwright, M. and G.J.F. Pugh, 1973. The effect of three fungicides on nitrification and ammonification in soil Soil Biol. Biochem., 5: 577-584.

Wainwright, M., 1987. Can fungi grow on fresh air? Mycologist, 21: 182-183.

Wainwright, M., 1988. Metabolic diversity of fungi in relation to growth and mineral cycling in soil: A review. Trans. Br. Mycol. Soc., 90: 159-170.

Wainwright, M., F. Barakah, I. Al-Turk and T.A. Ali, 1991. Oligotrophic micro-organisms in industry, medicine and the environment. Sci. Prog., 75: 313-322.