Hydroxamate Siderophore Content of Organic Fertilizers

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Abstract To estimate siderophore input into soil through fertilization, the hydroxamate siderophore content was determined in five organic fertilizers: granulated bacterial biomass (BAC); granulated fungal biomass (BIO); grape marc compost (BIV); chicken dung (ITA); and bacterial single cell protein biomass (PRU). Siderophore content, expressed as Desferrioxamine B equivalents as determined by a modified version of the Arthrobacter flavescens JG-9 bioassay, ranged from 0 to 20, 647 ng g^{-1} dry wt. of fertilizer (PRU < BIV < ITA < BAC < BIO). Recommended application rates of BIO would result in a calculated siderophore input of about 10 µg kg^{-1} of soil in the plow layer. Such additions could affect iron nutrition of plants and alleviate of iron chlorosis.

Keywords: Desferrioxamine B, Bacterial and fungal biomass, Chicken dung, Grape marc compost, Soil, Iron

INTRODUCTION

While main producers of hydroxamate siderophores are fungi and actinomycetes, some siderophores are released from bacteria. Sidreophores absorb onto organic soil material or possibly accumulate in soil micropores (Powell et al., 1980; Reid et al., 1984), and affect the iron nutrition of plants (Reid et al., 1986) This may not only increase yields, but may also diminish plant diseases (Schroth and Hancock, 1982; Kloepper et al., 1980; Elad, 1986; Schippers et all, 1986).

To assess whether and to what extent the application of fertilizers can lead to a siderophore input into soil we have determined the siderophore content of different organic fertilizers. For such analyses we have applied the Arthrobacter flavescens bioassay which we have modified to increase its sensitivity at low siderophore concentrations.

MATERIAL AND METHODS

Preparation of fertilizer extracts:

50 g of each fertilizer (see Table 1) were extracted for 1 h in 100 ml double distilled water at 4°C followed by centrifugation (5000 g, 20 min, 4°C). The supernatant was filtered through Schleicher & Schuell No. 595 1/2 before second centrifugation (28000 g, 20 min, 4° C). The extracts of grape marc compost (BIV), chicken dung (ITA) and bacterial single cell protein biomass (PRU) were evaporated under vacuum to about 1ml before double distilled water was added again to give a total volume of 5 ml. All extracts were filter sterilized (pore size of 0.22 µm) prior to storage at 4°C until the bioassay was carried out.

Bioassay:

Since most of the hydroxamate siderophores stimulate growth of Arthrobacter flavescens JG-9 (Powell et al., 1980; Lankford, 1973; Lochhead, 1958), this auxotroph was used to determine the hydroxamic acid content of different fertilizers in a plate assay. The assay was carried out as described by Estep et al. (1975) and Frederick et al. (1981) where filter paper discs (12.7 mm diameter; Schleicher & Schuell No. 740-E) are impregnated with 50 µl of samples or standard solutions.

In a modification of the assay a corkborer (6 mm outer diameter) was used to punch 3 wells (center distance between wells 48 mm) per petridish (94 mm diameter) into the nutrient medium. The wells were filled with 50 µl of samples or siderophore standards. As a standard, desferrioxamine B (DFOB, also known as desferrioxamine B methanesulfonate or :Desferal, Ciba-Geigy, CH-4000 Basel, Switzerland) was used in the following concentrations (ng ml^{-1}): 50, 100, 200, 400, 800, 1000, 2000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 20000.

Table 1: *Description of Organic Fertilizers*

FERTILIZER	Abbreviation used in text	Main* component	Producer
BACTOSOL	BAC	dried granulated bacterial biomass[1]	Biochemi GmbH, A-6250 KUNDL Austria
BIOSOL	BIO	dried granulated fungal biomass[1]	Biochemi GmbH, A-6250 KUNDL Austria
BIOVIN	BIV	grape marc compost[2]	Trever GmbH, A-2340 MOEDLING, Austria
ITALPOLLINA	ITA	chicken dung[1]	Italpollina S.P.A., I-37010 RIVOLI/ VERONA, Italy
PRUTEEN	PRU	bacterial biomass single cell protein[3]	ICI, BELLINGHAM, Cleveland, England

*Data on nutrient content of fertilizers; [1]Insam and Haselwandter, 1985; [2]Graefe, 1983, [3]Wainwright et al., 1985

For the regression lines (Fig. 1) the mean values (n=8) of growth zone diameters were plotted against the log of DFOB concentrations.

The data presented in Table 2 are the means of, at least, 4 replications of the assay. Student's t-test was applied to determine the significant differences between the means of the siderophore content of the fertilizers.

RESULTS AND DISCUSSION

Modification of the assay: well vs. filter disc application of solutions

The diameter of the growth zone surrounding the filter discs which were impregnated with 50 µl of the siderophore solutions were equal to those observed when 50 µl of the solutions were transferred into the wells. As demonstrated in Figure 1, the dose-response curves are identical. However, at low siderophore concentration the wells allow detection of even very small growth zones since the diameter of the wells is only 6 mm in comparison to 12.7 mm of the filter paper discs. It is also quicker and easier to carry out the well test as compared to the filter paper disc test, in particular as the sterile solutions is more complicated and time consuming.

Siderophore content of organic fertilizers

As shown in Table 2, the siderophore content of the organic fertilizers varied between 6 and 20,647 ng g^{-1} dry wt. With PRU no growth stimulation of Arthrobacter flavescens was detected; this indicates that this product which has been considered to

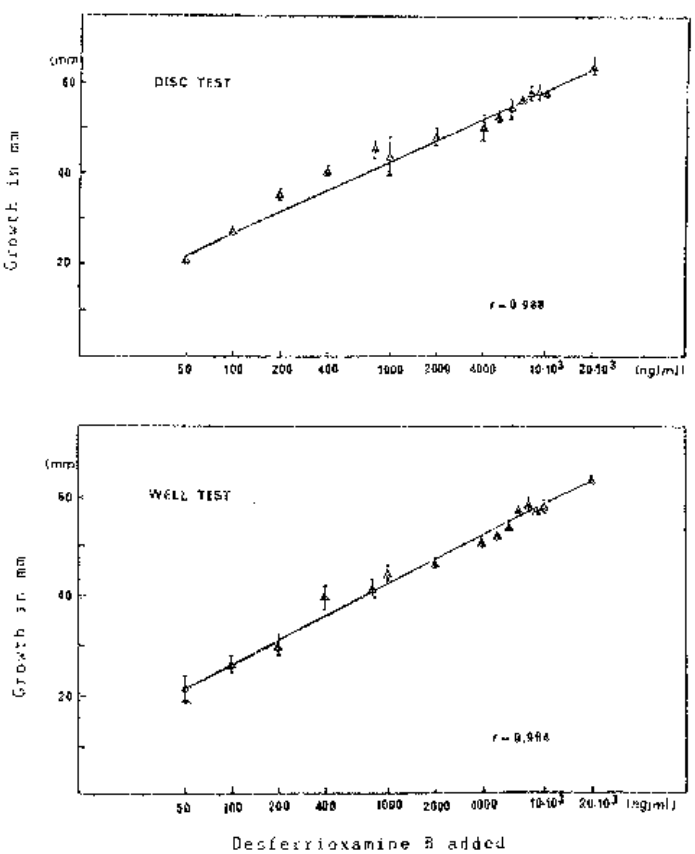

Fig 1: *Dose-reponse-curve for the Arthrobacter flavescens JG-9 plate bioassay; 50 ul of standard concentration (Desferrioxamine B) applied as impregnated filter paper discs (12,7 mm diamater) or in wells (6m mm diameter).*

Table 2: *Hydroxamate siderophore concentration in organic fertilizers.*

FERTILIZER	Desferrioxamine B equivalents (ng g^{-1} dry wt.)		
	$\bar{x}$	s_d	n
BAC	10224	+0	4
BIO	20647	+3579	4
BIV	14	+2	5
ITA	124	+22	7
PRU	not detected	-	4

have a fertilizer potential (Wainwright et al., 1983) does not contain sufficient amounts of water extractable siderophores to stimulate growth of the auxotroph. The catechol siderophore, enterochelin as well as the natural chelators citric acid, oxalic acid, and 2,3-dihydroxhbenzoic acid did not stimulate growth of A. flavescens JG-9 (Powell et al., 1980). Bossier and Verstraete (1986 a and b) also reported that Pseudomonas spp. produces siderophores that did not stimulate A. Flavescens JG-9. All data are significantly different from each other at P = 0.01. Abbreviations: $\bar{x}$ = mean value; s_d = standard deviation; n = number of replicates

Even when the fertilizer concentration in the water extract was raised by a factor of 20 as compared to BAC and BIO extracts (see Materials and Methods), A. flavenscens JG-9 stimulating compounds were not detected in PRU extracts. Evaporation of the extracts leading to a 20fold higher concentration, enabled us to detect, at least, some siderophores in ITA (124 ng g^{-1}) and BIV (14 ng g^{-1}). The bioassay might underestimate the siderophore concentration in BIV since this organic fertilizer contains humic and fulvic acids (Danneberg, 1982) which can reduce growth of A. flavenscens JG-9 in vitro (Bossier and Verstraete, 1986). In addition, it must be noted that, at least from soils, only a certain and variable, soil type specific percentage of hydroxamate siderophores can be extracted (Powell et al, 1980). This might also be of relevance for this study and requires further investigation.

Remarkably high concentrations of siderophores were found in BAC (10,224 ng g^{-1}) and BIO (20,647 ng g^{-1}). According to the recommendations of the producers the organic fertilizers BAC, BIO, BIV and ITA should be applied to the field at quantities in the range of 150 g m^{-2} (1500 kg ha^{-1}). In the case of BIO this leads to a siderophore input into soil of 3 mg m^{-2} (30 g ha^{-1}). Calculated on the basis of an assumption often made in agronomy that 1 has equals 3 x 10^6 kg of soil, the siderophore input is equal to 10 µg kg^{-1} soil. Such a siderophore input through fertilization is substantial if one considers that the siderophore concentration of soils is in the range of 0 to 150 µg kg^{-1} soil) as has been determined for a variety of soils applying the Arthrobacter bioassay or different methods (Akers, 1981; Harrington and Neilands, 1982; Powell et al., 1982; Reid et al., 1984; Bossier and Verstraete, 1986 a and b).

A siderophore addition to soil in the order of magnitude as mentioned for BIO can be expected to alleviate Fe nutrition deficiencies of plants when the level of available Fe in soil is low. And, indeed, it was observed that chlorosis of grape was drastically reduced after fertilization of the vineyard with BIO reduced after fertilization of the vineyard with BIO (800 kg ha^{-1} in the 1st year, 600 kg ha^{-1} in the 2nd year; Solar and

Lichtenegger, 1986). This possible micronutrient effect might also be important in explaining the observed increments in yield and quality improvement of the vine.

DISCLAIMER

Reference to a company and/or product is for purposes of information only, and does not imply approval or recommendation of the product.

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REFERENCES

Akers, H.A. 1981. The effect of waterlogging on the quantity of microbial iron chelators (siderophores) in soil. Soil Science 132:150-152.

Bossier, P. and W. Verstraete. 1986 a. Detection of siderophores in soil by a direct bioassay. Soil Biol. Biochem. 18:481–486.

Bossier, P. and W. Verstraete. 1986 b. Ecology of Arthrobacter JG-9-detectable hydroxamte siderophores in soils. Soil Biol. Biochem. 18:487–492.

Danneberg, O.H. 1982. Gefäßversuche zur Ermittlung des Düngerwertes von Traubentresterkomposten. Österreichisches Forschungszentrum Seibersdorf Rep.No.4149:112-128.

Elad, Y. 1986. Mechanisms of interactions between rhizospere microorganisms and soilborne plant pathogens. pp. 49–61. In: Microbial Communities in Soil. FEMS Symposium No.33. V. Jensen, A. Kjøller, L.H. Sørensen (eds.).

Estep, M., J.E. Armstrong and C. Van Baalen. 1975. Evidence for the occurrence of specific iron (III)-binding compounds in near-shore marine ecosystems. Appl. Microbiol. 30:186-188.

Frederick, C.D., P.J. Szaniszlo, P.E. Vickrey, M.D. Bentley and W. Shive. 1981. Production and isolation of siderophores from the soil fungus Epicoccum purpurascens. Biochemistry 20:2432-2436.

Graefe, G. 1983. Dünger und Energie aus Traubentrestern. Wirtschaftliche Nutzung eines Abfallproduktes in einem geschlossenen Stoffkreislauf. In: Bundesministerium für Wissenschaft und Forschung, Wien.

Harrington, G.J. and J.B. Neilands. 1982. Isolation and characterization of dimerumic acid from Verticillium dahliae. Journal of Plant Nutrition 5: 675-682.

Insam, H. and K. Haselwandter. 1985. Die Wirkung verschiedener Begrünungsmaßnahmen auf die mikrobielle Biomasse im Boden planierter Skipisten oberhalb der Waldgrenze. Zeitschrift für Bergtationstechnik 8: 23–28.

Kloepper, J.W., J.Leong, M. Teintze and M.N. Schroth. 1980. Enhanced plant growth by siderophores produces by plant growth-promoting rhizobacteria. Nature 286: 885-886.

Lankford, C.E. 1973. Bacterial assimilation of iron. Crit. Rev. Microbiol. 2:273-331.

Lochhead, A.G. 1958. Soil bacteria and growth-promoting substances. Bacteriol. Revs. 22:5-153.

Powell, P.E., P.J. Szaniszlo, G.R. Cline and C.P.P. Reid. 1980. Occurrence of hydroxamate siderophores iron chelators in soils. Nature 287:833-834.

Powell, P.E., P.J. Szaniszlo, G.R. Cline and C.P.P. Reid. 1982. Hydroxamate siderophores in the iron nutrition of plants. Journal of Plant Nutrition 5: 653-673.

Reid, R.K., C.P.P. Reid, P.E. Powell and P.J. Szaniszlo. 1984. Comparison of siderophore concentrations in aqueous extracts of rhizosphere and adjacent bulk soils. Pedobiologia 26:263–266.

Reid, C.P.P., P.J. Szaniszlo, and D.E. Crowley. 1986. Siderophore involvement in plant iron nutrition. pp. 29–42. In: T.R. Swinburne (ed.), Iron, Siderophores, and Plant Diseases. Plenum Publishing Corporation, NY.

Schippers, B., P.A.H.M. Bakker, A.W. Bakker, P.J. Weisbeek and B. Lugtenberg. 1986. Plant growth inhibiting and stimulating rhizosphere micro-organisms: 35–39, In: Microbial Communities in Soils. FEMS Symposium No. 33. V. Jensen, S. Kjøller, L.H. Sørensen (eds.).

Schroth, M.N. and J.G. Hancock. 1982. Disease suppressive soil and root-colonizing bacteria. Science 216: 1376–1381.

Solar, F. and E. Lichtenegger. 1986. Zustand und Melioration von Problemstandorten im Weinbau. Blick ins Land 21(11):1–4.

Wainwright, M., W. Nevell, and U. Skiba. 1985. Fertilizer potential of some commercially available forms of keratin and microbial biomass. Enzyme Microb. Technol. 7:108–110.