

# 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<sup>-1</sup> 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<sup>-1</sup> 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. Siderophores adsorb 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 al., 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 1 ml 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;

Table 1: Description of Organic Fertilizers

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

\*Data on nutrient content of fertilizers; <sup>1</sup>Insam and Haselwandter, 1985; <sup>2</sup>Graefe, 1983, <sup>3</sup>Wainwright et al., 1985

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<sup>-1</sup>): 50, 100, 200, 400, 800, 1000, 2000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 20000.

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  $\mu$ l of the siderophore solutions were equal to those observed when 50  $\mu$ l 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 n<sup>-1</sup> dry wt. With PRU no growth stimulation of *Arthrobacter flavescens* was detected; this indicates that this product which has been considered to

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

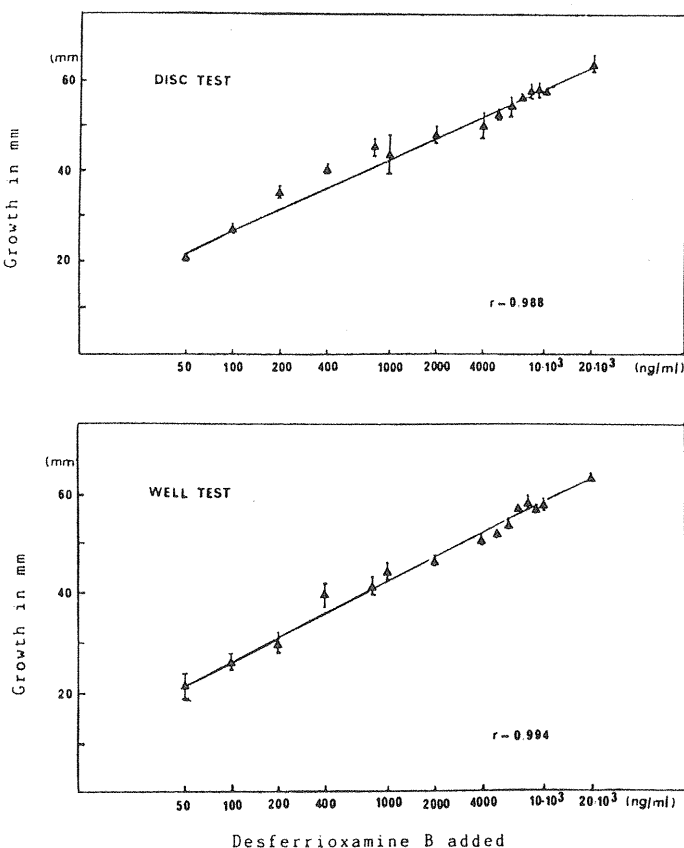
FERTILIZER	Desferrioxamine B equivalents (ng g <sup>-1</sup> dry wt.)		
	X	s <sub>d</sub>	n
BAC	10224	+ 0	4
BIO	20647	+3579	4
BIV	14	+ 2	6
ITA	124	+ 22	7
PRU	not detected	-	4

have a fertilizer potential (Wainwright et al., 1985) 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-dihydroxybenzoic 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<sub>d</sub> = 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. flavescens* 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<sup>-1</sup>) and BIV (14 ng g<sup>-1</sup>). 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. flavescens* 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<sup>-1</sup>) and BIO (20,647 ng g<sup>-1</sup>). 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<sup>-2</sup> (1500 kg ha<sup>-1</sup>). In the case of BIO this leads to a siderophore input into soil of 3 mg m<sup>-2</sup> (30 g ha<sup>-1</sup>). Calculated on the basis of an assumption often made in agronomy that 1 ha equals 3 x 10<sup>6</sup> kg of soil, the siderophore input is equal to 10  $\mu$ g kg<sup>-1</sup> 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  $\mu$ g kg<sup>-1</sup> 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<sup>-1</sup> in the 1st year, 600 kg ha<sup>-1</sup> in the 2nd year; Solar and



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

