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Evaluation of Nitrogen Fixation and Change of Microbial Flora in the Rhizosphere of Some Inulin Containing Plants

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Abstract
The present study investigates nitrogen fixation in the rhizospheres of four plant species; Dahlia pinnata, Helianthus annuus,Taraxacumofficinale and Cichoriumintybus with the change in their rhizosphere microbial flora. The four plant species contained a common polysaccharide, inulin in their roots. Nitrogen fixation was assessed by measurement of N_2-ase (C_2H_2) activity. Microbial activity was assessed by the production of carbon dioxide in rhizosphere soil samples. The relationship between the number of nitrogen fixing microorganisms and nitrogenase activity was observed, which also resemble the rate of CO_2 production. The different plant systems were found to have differences in the pattern of influencing the nitrogen microflora, although they contained a common polysaccharide substance in their roots. The N_2- ase (C_2H_2) activity was higher with inulin as an energy source than with glucose. Flowering plant (Dahlia pinnata) and weed plant (Taraxacumoficinale) were found to possess great potentiality in enriching the soil through asymbiotic nitrogen fixers compared to common cultivated plants like Cichoriumintybus. This study revealed that plants with inulin in their roots favoured the growth of free-living nitrogen fixing bacteria in soil, when used as an energy source resulted in higher nitrogenase activity compared with glucose, nitrogenase activity and numbers of free-living microorganisms as well as CO_2 production were closely related.

Keywords: *Inulin, nitrogen fixation, nitrogenase activity, rhizosphere, plant species.*

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I. Introduction

In special ecological situations such as the rhizosphere, the contribution of nitrogen fixing microorganisms might be enhanced if an accessible source of energy is made available to them. It is also known that the nitrogen fixation presumably depends primarily on the exudation pattern of each plant species[1].Several reports have demonstrated the possible contribution of free-living bacteria in increasing soil nitrogen-nitrite (NO_2) and nitrate (NO_3) through fixation of atmospheric nitrogen [2-6]. Nitrites and nitrates are major soil fertilizers. The natural fertility of a soil is mostly associated with nitrites and in particular, nitrates [7].

The exudation pattern of some plant species which contain inulin, a polysaccharide will be different from that of other species and will influence the nitrogen fixing bacteria in rhizosphere differently. The importance of this polysaccharide in biology and chemistry has been discussed many times and it has been proposed that frutans, including levans, play a dominant role in the chemical changes which occur during the life of plants. The presence of these compounds has been correlated with resistance to frost [8]. In earlier study, Vlassak and Jain[9] have found significant N_2-ase (C_2H_2) activity in two inulin containing plants, *Cichoriumintybus*and*Taraxacumofficinale.* The objective of this study was to evaluate nitrogen fixation and change of microbial flora in the rhizosphereof some inulin containing plants.

II. Materials and Methods

Growth of plants and collection of rhizophere samples

Four plant species such as *Cichoriumintybus, Taraxacumofficinale, Helianthus annuus* and *Dahlia pinnata* were selected and grown in the greenhouse in pots and under conditions as described earlier by Jain and Vlassak[10]. Rhizosphere samples were collected by carefully removing the soil closely adhering to the roots and other extraneous debris (pebbles, rootlets), sieved and ground to fine powder after air drying. This soil was called rhizosphere soil sample whereas the soil from the bare pots was called non-rhizosphere soil sample.

Determination of nitrogenase activity of rhizosphere samples

The treatment procedurefollowed was that described by[10]. Appropriate controls without an energy source were also analyzed. Moisture content equivalent to 150% of water holding capacity (WHC) was maintained. The nitrogenase activity was measured at two-hourly intervals by injecting 1ml gas samples from each flask into a Hewlett Packard Gas Chromatograph with a Porapak R. column of 2m. For CO_2 estimation the samples were prepared as described for nitrogenase activity assays, except that no acetylene was added to the flasks. From the airtight flasks incubated at 29^{o}C, 1ml of each gas sample was injected at 48h into a Hewlett Packard Gas Chromatograph with a Porapak R. Column of 2m.

Bacteriological analysis

Bacterial counts were made on soil yeast extract agar (SYEA) medium and on nitrogen free agar medium [11]. The soil samples were also analyzed for *Azotobacter* using plate counts on Jensen's agar medium; for *Pseudomonas*,using nutrients agar (beef extract, 3.0g; peptone 5.0g; agar 15.0g; H_2O 1000 ml supplemented with centrimide 10g) and for members of enterococci using Mac Conkey's agar medium (agar 10g, sodium thioglycolate, 5g; peptone 20g; sodium chloride 5g; and H_2O 1000ml), *Clostridium* were counted by the most probable number (MPN).

III. Results

The results of nitrogenase activity in rhizosphere soil of some inulin containing plants are presented in Table no 1. The results showing the effect of microbial populations on nitrogen fixation in the pots are presented in Table no 2. The microbial population in relation to CO_2 production and nitrogenase activity is succinctly illustrated in **Figureno1**.

Table no 1. Nitrogenase activity in the rhizosphere of some inulin containing plants (nmoles $C_2H_4h^{-1}g^{-1}$ of soil.)

		Age of plants in days						
Crop	Treatment	0	30	60	90	120	150	180
Bare pots	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Glucose	23.0	15.1	8.5	9.1	8.5	0.1	0.0
	Inulin	38.0	26.4	14.2	10.5	10.7	0.9	0.0
Dahlia	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
pinnata	Glucose		28.5	22.2	68.4	46.8	45.4	32.8
	Inulin		28.1	23.0	75.4	67.0	56.5	35.2
Helianthus annus	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Glucose		15.1	10.9	23.4	30.2	22.3	8.3
	Inulin		15.5	13.2	23.4	57.2	41.4	15.9
Taraxacumofficinale	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Glucose		22.1	13.3	77.0	38.2	37.5	57.6
	Inulin		26.4	13.1	96.0	42.4	40.6	52.0
Cichoriumintybus	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Glucose		18.1	11.1	36.4	26.9	12.6	1.6
	Inulin		26.1	16.0	57.1	35.8	24.15	5.1

S.E (m): ±1.4 days, treatments ±1.3
C.D at 5%: 3.9 days; C± 3.6

Table no 2. Microbial analysis of rhizosphere soil of some inulin containing plants (numbers of organisms per g (CFU/g) of soil.

		Age of plants in days					
Soil from	Analysis for	0	30	60	90	120	150
Barepots (non-rhizosphere)	Total Bact. counts x10^5	28.0	36.0	18.0	10.0	6.0	5.4
		40.0	34.0	18.0	8.0	4.0	1.6
	TBC on NF media x10^4	30.0	0.6	0	0	0	0
		18.0	11.0	6.0	4.5	2.6	2.6
	Azotobacter	2.0	0.7	0	0	0	0
	Clostridium x10^2						
	*Enterococci*x10^2						
Dahlia pinnata	TBC x10^5		50.0	37.0	330.0	180.0	93.0
	TBC on NF media x10^4		54.0	42.0	189.9	180.0	109.0
	Azotobacter		3400	1000	30000	21000	1800
	*Clostridium*x10^2		19.0	9.0	170.0	60.0	36.0
	*Enterococci*x10^2		25.0	4.0	38.0	0	0
Helianthus annus	TBC x10^5		27.0	26.0	94.0	170	88.5
	TBC on NF media X10^4		48.0	36.0	56.0	980	50.0
	Azotobacter		10.0	10.0	90.0	180.0	80.0

	Clostridium x10^2	6.0	4.0	7.0	30.0	6.0
Taraxacumofficinale	TBC x10^5	68.0	39.0	280	229.0	232.0
	TBC on NF media x10^4	52.0	44.0	169.0	104.0	110.0
	Azotobacter	103.0	20.0	35.0	180.0	236.0
	*Clostridium*x10^2	18.0	6.0	35.0	9.0	18.0
	*Enterococci*x10^2	2.6	2.0	34.0	8.0	6.0
Cichoriumintybus	TBC x10^5	34.0	21.0	146	70.0	35.0
	TBC on NF media x10^4	50.0	34.0	139	96.0	28.0
	Azotobacter	20.0	5.0	54.0	30.0	0
	*Clostridium*x10^2	11.0	4.5	31.0	16.0	6.0
	*Enterococci*x10^2	2.0	1.0	8.0	3.0	0

Legend: TBC = Total bacterial count; CFU = Colony forming unit

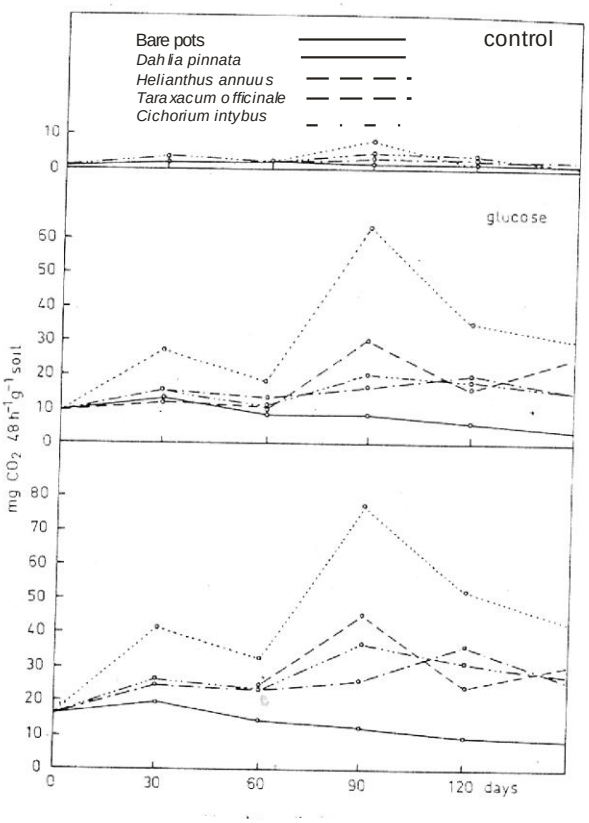

Figure no1. Production of CO_2 in the rhizosphere soil containing plants. SE. (m): ± 1.6 days: treatment ± 1.6. C.D. at 5%: ± 4.6 days: treatments ± 4.5

IV. Discussion

The nitrogenase activity in rhizosphere soil was measured in anaerobic conditions and the results are presented in Table no 1. It is very clear from the table that there is no activity in all the experiments when no energy is supplied. With regard to the bare pots it is shown that the soil has initial nitrogenase activity equivalent to 23 n moles $C_2H_4g^{-}h^{-1}$ when glucose was supplied and 38 nmoles C_2H_4 with inulin as carbon source. In these pots, the nitrogenase activity decreases steadily and continuously. This can be explained by the fact that during the experimental time organic nitrogen is mineralized and ammonia-N and nitrate-N accumulate in the soil, because it is not taken up by plants[9]. It is known that there is a reverse relationship between nitrogenase activity and mineral nitrogen content in the soil [9].

The number of microorganisms was closely related to nitrogenase activity since the maximum counts were observed when nitrogenase activity was high. In bare pots the microbial population and nitrogenase activity decreased steadily. As regards CO_2 evolution, the microorganisms respired more in the presence of inulin in all the treatments in comparison with glucose, although the rate of CO_2 production decreased continuously **(Figureno 1).** The maximum N_2-ase (C_2H_2) activity in the rhizosphere of *Dahlia, Taraxacum* and *Cichorium* was seen at 90 days, after which it declined. Conversely, in the bare pots with inulin, the activity was

higher than with glucose. In the control, without inulin and glucose as a carbon source, no nitrogenase activity was detected. Again, the numbers of microorganisms were related to the nitrogenase activity in anaerobic condition. When the nitrogenase activity increased, as in the case of *Taraxacum*, there was also a corresponding increase in the numbers of micro-organisms, particularly the nitrogen fixing organisms.

The production of CO_2 showed a similar trend. In contrast to other plant systems, the maximum N_2-ase (C_2H_2) activity in *Helianithus*sp. was observed when plants were 120 days old. Here the activity obtained with inulin was again higher than with glucose. The microbial population was also increased in 120 days and their activity in terms of production of CO_2 was also higher at this time with both glucose and inulin respectively. However, production of CO_2 in the control slightly decreased at 120 days than at 90 days.

The developments of *Azotobacter* and *Clostridium* populations in the rhizosphere of *Dahlia* was of special significance. It has been reported that some dominant nitrogen fixers like *Clostridium* are able to use inulin as their energy source and hence they could multiply in great numbers and consequently a high N_2-ase (C_2H_2) activity could be expected. The N_2-ase (C_2H_2) activity in the *Dahlia*rhizosphere was second highest,the highest being *Taraxacumofficinale.* But the microbial flora of *T. officinale*particularly the *Azotobacter* and *Clostridium* populations was far less than that in *Dahlia*. This difference in the N_2-ase (C_2H_2) activity could be attributed to effectiveness of bacterial strains rather than to number of organisms.

The N_2-ase (C_2H_2)activity in *Helianthus* may have been affected by the poor growth of the plants from the beginning. One of the explanations was that*Helianthus* might require a rich soil for its establishment as the soil provided was poor and

contained sand in 1:1 ratio (W/w). This became apparent when plant growth was enhanced after fertilization with phosphate monopotassium (1g/pot), and then maximum N_2-ase (C_2H_2) activity was obtained at 120 days. The increase in N_2-ase (C_2H_2) activity in *T.officinale* again in 180 days was accompanied by new growth of the plants along with the increase in microbial population and microbial activity in terms of CO_2 production. These data confirm the observations in intact plant systems. The maximum nitrogenase activity was observed when the plants were in full vigour and flowing stage [8,12]. These data also support earlier findings where *T.officinale* had higher N_2-ase (C_2H_2) activity than *C. intybus*[9]. This confirms the importance of *T. officinale* in enriching the soil through nitrogen fixation.

V. Conclusions

This studyrevealed that (1), plants with inulin in their roots favoured the growth of free-living nitrogen fixing bacteria in soil (2), its (inulin) use as an energy source resulted in higher nitrogenase activity compared with glucose and (3), nitrogenase activity and numbers of free-living microorganisms and CO_2 production are closely related. The data also confirms the efficacy of these plants in enriching the soil by nitrogen fixation.

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