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Diversity in Rhizosphere Fungi of Local Crop Plants around Bhavnagar (GUJ) and their Siderophore Production

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About twenty fungi had been isolated from rhizosphere and rhizoplane of four local crop plants as cotton, banana, tuver and brinjal from agricultural soils at Bhavnagar, out of which 10 belonged to genus *Aspergillus*, 3 to *Penicillium* and 3 to *Fusarium* while, 4 isolates were unidentified. Diversity was assessed using several indices for relative frequency, eveness, richness, shannon's and similarity indices. Based on relative frequency *Aspergillus* sp. was the predominant microflora at the four crop plants while, *Fusarium* sp. could be grouped as just present species. Brinjal was the most diverse and it represented maximum evenness while, with respect to richness it was least. Except 3, all the isolates produced siderophores that were trihydroxamate hexadentates. Quantification of siderophores revealed *Fusarium* sp. 3 from cotton to be the maximum siderophore producer (468 μg/ml) while, *Aspergillus* sp. 7 from banana the least (47 μg/ml). Thus, even though *Aspergillus* spp. was the predominant microflora at all the 4 crop plants, *Fusarium* spp. were the potential siderophore producers. Hence, irrespective of the predominant *Aspergillus* spp. in the rhizosphere, *Fusarium* spp. can prove to be potential candidates for plant growth promotion. The present study thus, does not indicate any relationship between the dominant microflora present in the rhizosphere and their ability to promote plant growth through siderophore production.

Key words: Rhizosphere, Biocontrol, Diversity, Siderophores, Trihydroxamate hexadentates.

The term "rhizosphere" coined by Hiltner[1] (1904), is defined as a zone of microbial proliferation in and around roots[2]. The rhizosphere has attracted much interest as it is a habitat in which several biologically important processes and interactions take place. It is populated by a diverse range of microorganisms as bacteria and fungi that both colonize this habitat and hence are called rhizobacteria and rhizosphere fungi respectively[3].

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The interaction between biocontrol fungi and fungal pathogens in the rhizosphere is the focus of scientific research, on par with work on bacterial – fungal plant pathogen interactions. However, there is an extra dimension in the quality of the interactions with fungi as they have much greater potential than bacteria to grow in the rhizosphere through their hyphal growth[4]. A variety of fungi are known to act as bio-control agents. They act by mechanisms as competition for nutrients *viz* iron through siderophores, antibiosis, mycoparasitism or direct growth promotion. However, competition for iron has been accepted as one of the most widely used strategy for bio-control mechanism.

Despite the fact that iron is the 4th most abundant element in the earth's crust, it is unavailable to microorganisms and plants, as it forms insoluble stable complexes of ferric

oxyhydroxide (FeOOH) at neutral to alkaline pH of the soil in which the earth abounds[5]. Hence, both microorganisms and plants have evolved three sophisticated strategies to overcome the deficiency of iron i.e., chelation, reduction and protonation. Among these, chelation through siderophores is the most widely used strategy[6].

In spite of the reports of importance of fungi in ecological systems, systematic data on fungal diversity from rhizosphere and rhizoplane of crop plants and their siderophores are scanty. We present an account of (i) the diversity of fungi from rhizosphere and rhizoplane of local crop plants at Bhavnagar (ii) their siderophore production and (iii) to observe relationship if any between fungal diversity and plant growth promotion through siderophore production.

MATERIAL AND METHODS

Sample collection

For isolation of fungi from rhizosphere soils at Bhavnagar, local crop plants as Cotton *(Gossypium arboretum),* Banana *(Musa paradisiacal), Tuver* (*Cajanus indicus)* and Brinjal (*Solanum nigrum)* were selected. 0.1g of loosely adhering soil to the roots was taken in 10ml of sterile distilled water and shaken for 10- 20 min. in a cyclomixer to get uniform soil suspension. Similarly, 0.1 g roots with tightly adhering soil particles were cut into small pieces, taken in 10 ml of sterile distilled water and shaken for 10-20 min to get uniform rhizoplane suspension.

The soil suspensions thus, obtained from rhizosphere and rhizoplane samples were diluted to 10^{-3} and used for the isolation of rhizosphere and rhizoplane fungi by dilution plate method. The samples were streaked onto potato dextrose agar (PDA), supplemented with 100mg /ml of streptomycin to avoid bacterial growth and incubated at 30 º C. The isolates obtained were purified by repeated sub-culturing and stored at 4 º C until use.

Diversity indices

Relative frequency (RF)

$$(RF) = \frac{\text{No. of samples in which species is present}}{\text{Total No. of samples}} \quad 100$$

Based on the values of occurrence, RF was interpreted as 0-25 % (just present); 26-50 % (common constituents); 51-75 % (important) and 76-100 % (dominant).

Similarity Index

Similarity index (SI) to assess similarities between the crop plants with respect to isolates was calculated[2].

$$SI = \frac{2C}{A+B}$$

A and B are the number of varieties obtained from rhizosphere and rhizoplane samples between crops and C is the number of varieties common to all the crops.

Shannon's diversity index

Shannon Weiner diversity index was calculated to assess the diversity of sites for all the four crops[7].

$$H' = -E\ pi\ (\ln pi)$$

p is the ratio of the number of isolates of i^{th} variety to the sum of isolates at crop plants.

Evenness and Richness

As diversity is composed of two elements (i) evenness and (ii) richness, evenness (E) was calculated using E_{pielou} equation[8] and richness (R) was calculated using $R_{menhinick}$[7]

$$R_{pielou\ menhinick} = \frac{H'S}{Ln\ (S)n}$$

H' is Shannon Weiner index, S is number of varieties and n is total number of isolates obtained from a crop plant.

Detection of siderophore production

Media for siderophore production

Grimm- Allen medium[9] that contained (g/ L of distilled water) K_2SO_4, 1; K_2HPO_4 , 3; ammonium acetate,3; citric acid, 1; sucrose, 40; *p*H- 6.8 (with ammonia) was used. The medium was supplemented with Thiamine, 2 mg; $CuSO_4.5H_2O$, 0.005 mg; $MnSO_4.H_2O$, 0.035 mg; $ZnSO_4.7H_2O$, 2 mg; $MgSO_4.7H_2O$, 80 mg. The medium was decontaminated of iron by adding 8 – hydroxyquinoline dissolved in chloroform. When shaken, this formed ferrous or ferric hydroxyquinates. After separation, the chloroform layer was removed and the medium was washed

repeatedly with chloroform to ensure complete removal of iron complexes and any residual 8 – hydroxyquinoline which could inhibit growth[7]. 150 ml medium was dispensed in 500ml conical flasks.

Iron decontamination

All glass wares were soaked overnight in 6M HCL and rinsed with distilled water several times to remove traces of iron.

Screening for siderophore production

$FeCl_3$ test

In $FeCl_3$ test, a red to purple colour is generated upon coordination of the metal ion with the siderophore. To 1ml of culture filtrate was added 1 - 5ml of 2% ferric chloride solution. The formation of red or purple colour indicated the presence of siderophores. The ferric complex was easy to follow for its red colour compared to deferricompounds[10].

Chrome azurol sulphonate (CAS) assay

It is a universal test for the detection and determination of siderophores as 0.02 µM of siderophores can be determined. CAS assay is based on the observation that when a strong chelator (e.g. a siderophore) removes iron from CAS / iron / detergent complex, the release of free dye is accompanied by change in colour from blue to orange. The dye can thus be used in solution or can be incorporated into agar plates. A 6ml volume of 10 mM HDTMA was placed in a 100ml volumetric flask and diluted with water. A mixture of 1.5ml iron (III) solution (1mM $FeCl_3$. $6H_2O$ in 10mM HCl) and 7.5ml of 2mM aqueous CAS solution was slowly added with stirring. A 4.307g quantity of anhydrous piperazine solution was dissolved in water and 6.25ml of 12M HCl was carefully added. This buffer solution was rinsed in a volumetric flask, which was then filtered to afford 100ml of CAS solution. To 1ml of supernatant, was added 1ml of CAS solution. After 2 - 4 min., the colour changed from blue to orange indicating siderophore production. The solutions were stored in the dark[11].

CAS agar plate test

At 10µM Fe (III) concentrations, the agar develops a rich blue colour. The siderophores excreted by iron-starved microorganisms generally exceeds this level. This results in a complete change to orange colour.

To prepare 1L of blue agar, 60.5mg CAS was dissolved in 50ml of distilled water and mixed with 10ml of iron (III) solution (1mM $FeCl_3$. $6H_2O$ in 10mM HCl) with stirring. This solution was slowly added to 72.9mg HDTMA dissolved in 40ml water. The resultant dark blue liquid was autoclaved. Also, autoclaved was a mixture of 750ml H_2O, 100ml (10×mM9 salts), 15g agar, 30.24g PIPES, 12.0g of a 50% (w/w) NaOH solution to raise the *p*H of PIPES (6.8) and 25% NaCl. After cooling to 50°C, 30ml of filter - sterilized casamino acids (10%), a carbon source and other required supplements, like vitamins and antibiotics, were added to sterile solution, along the glass wall, with enough agitation to achieve mixing without generation of foam. Each plate received 30ml of blue agar. Interaction of the dye with agar extends the applicability of the method to higher *p*H values, although a change to green was observed above *p*H 7[11].

Chemical characterization of siderophores

Detection of hydroxamate, catecholate and carboxylate nature of siderophores.

Chemical assays

15 day old culture filtrates were used to examine for hydroxamate nature by $FeCl_3$ and Tetrazolium tests, catecholates by Arnow's and $FeCl_3$ tests, while carboxylate nature was determined by spectrophotometric test.

Detection of hydroxamate siderophores

$FeCl_3$ test

To 1ml of culture filtrate was added 1 – 5ml of freshly prepared aqueous 2% $FeCl_3$ solution. The formation of orange colored ferric hydroxamate, showing maxima at 420 - 450nm indicated presence of hydroxamate siderophores[12].

Tetrazolium test

This test is based on the capacity of hydroxamic acids to reduce tetrazolium salt, by hydrolysis of hydroxamate group in presence of strong alkali[13].

To a pinch of tetrazolium salt, were added 1 - 2 drops of 2N NaOH and 1ml of the test sample. Instant appearance of a deep red color indicated presence of hydroxamate siderophore.

Detection of catecholate siderophores

Arnow's test

Catecholate siderophores on reaction, in succession with nitrous acid, molybdate and alkali, yield a pink chromogen that absorbs maximally at 515nm. To 1ml of culture filtrate was added 0.1ml of 5N HCl, 0.5ml of Arnow's reagent containing

10g each of $NaNO_2$ and $Na_2MoO_4.2H_2O$ in 50ml distilled water. A yellow colour results at this point. 0.1ml of 10N NaOH (a red colour results) and enough distilled water was added to make the volume to 5ml. Absorbance was noted at 515nm and quantified using 2, 3 - dihydroxybenzoic acid as standard[14].

$FeCl_3$ test

Catecholate siderophores form a wine coloured complex with λmax at 495nm, on addition of ferric chloride. To 1ml of culture filtrate was added 1ml of 2% $FeCl_3$. The formation of wine colored complex was monitored at 495nm[12].

Detection of carboxylate Siderophores

Spectrophotometric test

To the culture filtrate was added 1ml of 250μM $CuSO_4$ and 2ml of acetate buffer at *p*H 4. The copper complex formed was observed for absorption maximum between 190-280 nm. There is no specific wavelength at which the copper complex gets absorbed. The entire wavelength 190 – 280nm was scanned to observe the peak of absorption of siderophore[15].

Bioassays

Specific indicator strains were used for the various types of siderophores as *Arthrobacter flavescens* JG9 for hydroxamates, *Salmonella typhimurium* enb7 for catecholates and *Morganella morganii* SBK3 for carboxylates. (*A. flavescens* JG9 and *S. typhimurium* enb7 were kindly supplied by Sally Leong, USA and *M. morganii* SBK3 was supplied by Rolf Reisbrodt, Germany)[16].

The indicator strains, which are non-siderophore producers, were iron - starved and seeded on AM medium. The culture filtrate of test organisms were loaded on filter paper discs and deftly placed on the surface of the AM medium. After incubation, formation of visible growth surrounding the disc indicated siderophore production by the test organism.

Quantification of siderophores

To 1ml of culture filtrate was added 1ml of CAS assay solution[17] and the A_{630} was read after 1h of incubation at room temperature[18,19]. Simultaneously, 1ml of CAS assay solution was also added to standard compounds desferrioxamine mesylate (DFOM) (for hydroxamate), 2,3 dihydroxybenzoic acid (for catecholate), and rhizoferrin (R- for carboxylate) siderophores. Rhizoferrin was kindly supplied by Gunther Winkelmann, Germany. The CAS activity of standard compounds was also read at 630nm, after 1h of incubation. Reference solution contained 1ml of uninoculated medium to which was added 1ml of CAS solution. The amount of siderophore production was extrapolated from the standard graph and denoted as DFOM (μg/ml), DHBA (μg/ml) and RE (μg/ml) for hydroxamate, catecholate and carboxylate siderophores respectively.

RESULTS AND DISCUSSION

About twenty fungi had been obtained from rhizosphere and rhizoplane of four crop plants at diverse sites out of which 6 were from cotton, 5 from banana, 5 from tuver and 4 from brinjal. Thus, maximum numbers of fungal isolates were obtained from cotton (6) and least from brinjal (2). Based on their colony characteristics, pigmentation and morphology, 10 isolates belonged to genus *Aspergillus (*designated as *Aspergillus* spp. 1-10*)*, 3 to *Penicillium (Penicillium* spp. 1-3*)*, and 3 to *Fusarium (Fusarium* spp. 1-3) while, 4 that could not be identified were designated as UN F spp. 1-4.

Relative frequency (RF), Similarity index (SI), Shannon's diversity index, Richness (R) and Evenness (E) had been calculated using various indices. On the basis of gradation of Relative frequency, the distribution pattern of the isolates at various crop plants could be arranged as (Table 1)

crop 1 > crop 3 > crop2 > crop 4

Moreover, relative frequency suggested aspergilli to be prevalent among all the four crops plants. It was dominant in rhizosphere and rhizoplane of cotton while, it constituted important species of banana and brinjal. On the basis of relative frequency *Aspergillus* spp. could be grouped as important species, *Penicillium* as common while *Fusarium* spp. and Unidentified species (Un F1 – Un F4) as just present.

Similarity matrix between four crop plants using Sorensen coefficient index (Table 2) indicated maximum similarity in the isolates between crop plants banana - tuver, least was between banana - brinjal while, dissimilarity was observed between cotton – brinjal, tuver – brinjal . Based on similarity and matrix of the isolates, crop plants could be arranged as.

crop 2–3 > crop 1-2 > crop 1-3 > crop 2-4.

Diversity, Richness and Evenness have been calculated by Shannon's diversity index (H'), Richness as R_{max} and Evenness as $E_{pielous}$ indices (Table 3). Brinjal represented maximum fungal diversity and evenness (3.01), cotton showed least (1.61). However, with respect to richness cotton showed maximum richness while brinjal and tuver the least. Thus, cotton exhibited minimum diversity and evenness, but with respect to richness, it was more.

Screening the isolates for siderophore production suggested (Table 4) that except 3 (*Aspergillus* sp. 6, 8 and UN F. sp. 4), all the test isolates produced siderophores as evidenced by positive $FeCl_3$ test, CAS assay and CAS agar plate test. Chemical nature of siderophores as hydroxamate, catecholate, carboxylate or mixed type (Table 5) revealed the siderophores to be hydroxamate in nature. Hydroxamate nature of siderophores was further confirmed by Bioassays that are more sensitive than chemical assays (Neilands 1984), using indicator organisms *Arthrobacter flavescens* JG 9 for hydroxamate, *Salmonella typhimurium* enb 7 for catecholates and *Morganella morganii* SBK 3 for caboxylate siderophores (Table 6). The siderophores of the test isolates produced growth zones with indicator organisms *A. flavescens* JG9, indicating their hydroxamate nature. However, they did not

Table 1. Relative frequency (RF) of fungal isolates from rhizosphere and rhizoplane of crops plants

Test isolates	Cotton %	Banana %	Tuver %	Brinjal %	RF Value %
Aspergillus spp.	78	60	35	65	70
Penicillium spp.	5	30	30	Nil	40
Fusarium spp.	6	25	Nil	Nil	20
Un F1	5	-	-	-	-
Un F2	-	8	-	2	5
Un F3	-	-	-	10	10
Un F4	5	-	5	-	10

Un F: Unidentified fungi; Grade I: (0-25) Just present; II: (26-50) common Species; III: (51-75) Important species; IV: (76-100) Dominant Species. – indicates absence of isolates

Tables 2. Similarity matrix between four crop plants

Crops	Cotton	Banana	Tuver	Brinjal
Cotton	1.00	0.54	0.44	0.00
Banana		1.00	0.56	0.25
Tuver			1.00	0.00
Brinjal				1.00

Table 3. Indices representing diversity, richness and evenness

Crops	Number of varieties	Shannon Weiner Diversity (H')	$R_{Menhinick}$	E_{Pielou}
Cotton	6	0.90	0.44	1.61
Banana	5	1.58	0.42	2.55
Tuver	3	2.61	0.23	2.87
Brinjal	3	2.74	0.24	3.01

produce growth zones with *S. typhimurium* enb7 and *M. morganii* SBK3 ruling out their catecholate and carboxylate nature.

On the basis of shift in ëmax and stable/ unstable colour of ferric hydroxamates at pH 4-9, siderophores were characterised as

Table 4. Screening of rhizosphere fungi for siderophore production

Test Isolates	$FeCl_3$ Test	CAS Test	CAS agar plate assay
Aspergillus sp. 1	+	+	+
Aspergillus sp. 2	+	+	+
Aspergillus sp. 3	+	+	+
Aspergillus sp. 4	+	+	+
Aspergillus sp. 5	+	+	+
Aspergillus sp. 6	-	-	-
Aspergillus sp. 7	+	+	+
Aspergillus sp. 8	-	-	-
Aspergillus sp. 9	+	+	+
Aspergillus sp. 10	+	+	+
Penicillium sp. 1	+	+	+
Penicillium sp. 2	+	+	+
Penicillium sp. 3	+	+	+
Fusarium sp. 1	+	+	+
Fusarium sp. 2	+	+	+
Fusarium sp. 3	+	+	+
UN F 1	+	+	+
UN F 2	+	+	+
UN F 3	+	+	+
UN F 4	-	-	-

Table 5. Detection of chemical nature of siderophores. (+ positive, - negative)

	$FeCl_3$ Test		Tetrazolium Test	Arnow's Test	Shenker's Test
	Peak at (420-450nm)	Peak at (495nm)			
Test Isolates	(hydroxamate)	(catecholate)	(hydroxamate)	(catecholate)	(carboxylate)
Aspergillus sp. 1	+	-	+	-	-
Aspergillus sp. 2	+	-	+	-	-
Aspergillus sp. 3	+	-	+	-	-
Aspergillus sp. 4	+	-	+	-	-
Aspergillus sp. 5	+	-	+	-	-
Aspergillus sp. 7	+	-	+	-	-
Aspergillus sp. 9	+		+		-
Aspergillus sp.10	+	-	+	-	-
Penicillium sp. 1	+	-	+	-	-
Penicillium sp. 2	+	-	+	-	-
Penicillium sp. 3	+	-	+	-	-
Fusarium sp. 1	+	-	+	-	-
Fusarium sp. 2	+	-	+	-	-
Fusarium sp. 3	+	-	+	-	-
UN F 1	+	-	+	-	-
UN F 2	+	-	+	-	-
UN F 3	+	-	+	-	-

trihydroxamate hexadentates (Table7).

The amount of hydroxamate siderophore production was extrapolated from the standard curve using desferioxamine mesylate (DFOM) as standard compound and quantified as DFOM µg/ml (Table 8). *Fusarium* sp. 3 isolated from cotton was maximum siderophore producer (468 µg/ml) followed by *Fusarium* sp. 2 (346 µg/ml) and *Aspergillus* sp. 7 isolated from banana was the least (47 µg/ml).

Microbial diversity constitutes the most extraordinary reservoir of life in the biosphere that we have just begun to explore and understand. In the rhizosphere that is on the plant root or its close vicinity bacteria are abundantly present, most often organised in micro colonies. These bacteria called as rhizobacteria not only benefit from the nutrients secreted by the plant roots but also beneficially influence the plant in a direct or indirect way, resulting in stimulation of its growth[20]. Competition for nutrients as iron has been widely accepted as one of the factor for stimulating plant growth[21]. However, the diversity of rhizosphere fungi and their role in competition for nutrients viz. Fe^{3+} through siderophores, has comparatively received less attention. Hence, we have attempted to access the diversity of rhizosphere fungi around local crop plants and examined their siderophores production. *Aspergillus* spp. was found to be dominant among the rhizosphere of all the crop plants. However, the dominance of *Aspergillus, Penicillium* and *Paecilomyces* spp. in extreme environment as has been reported[22].

Diversity is composed of two elements: richness and evenness, so that highest diversity occurs in the communities, with many different species present in relatively equal abundance (evenness)[23]. The present results are in agreement to these findings but are in contrast to richness. Rhizosphere of cotton represents maximum richness, but with respect to diversity and evenness it is the least.

Non- production of siderophores by the 3 isolates may be due to their origin in acidic soils or as in lactobacilli that has replaced Fe^{3+} with Mn and Co. *Saccharomyces cerevisiae* and, *Neisseria* spp. have been reported to reduce Fe^{3+} outside the cell due to ferric reductases thus, obviating the

Table 6. Bioassays of siderophore using indicators strains for hydroxamate catecholate and carboxylate nature (+ positive, - negative)

Test Isolates	*Arthrobacter flavescens* JG9 (hydroxamate)	*Salmonella typhimurium e*nb7 (catecholate)	*Morganella morganii* SBK3 (carboxylate)
Aspergillus sp. 1	+	-	-
Aspergillus sp. 2	+	-	-
Aspergillus sp. 3	+	-	-
Aspergillus sp. 4	+	-	-
Aspergillus sp 5	+	-	-
Aspergillus sp. 7	+	-	-
Aspergillus sp. 9	+	-	-
Aspergillus sp. 10	+	-	-
Penicillium sp. 1	+	-	-
Penicillium sp. 2	+	-	-
Penicillium sp. 3	+	-	-
Fusarium sp. 1	+	-	-
Fusarium sp. 2	+	-	-
Fusarium sp. 3	+	-	-
UN F 1	+	-	-
UN F 2	+	-	-
UN F 3	+	-	-

Table 7. Mono-, di-, and trihydroxamate nature of siderophores and their ligand denticity

Test Isolates	Different pH	$FeCl_3$ peak OD at 420- 450 Nm	λmax shift	Inference	Colour	Inference
	4	424				
	5	425				
Aspergillus sp. 1	6	420				
	7	425	12	Trihydroxamate	LightBrown	Hexadentate
	8	432				
	9	430				
	4	420				
	5	420				
Aspergillus sp. 2	6	420				
	7	421	2	Trihydroxamate	Brown	Hexadentate
	8	421				
	9	422				
	4	415				
	5	415				
Aspergillus sp. 3	6	415				
	7	417	6	Trihydroxamate	Brown	Hexadentate
	8	417				
	9	421				
	4	420				
	5	420				
Aspergillus sp. 4	6	419				
	7	419	2	Trihydroxamate	Reddish	Hexadentate
	8	418				
	9	418				
	4	436				
	5	440				
Aspergillus sp 5	6	444				
	7	437	9	Trihydroxamate	Reddish	Hexadentate
	8	443				
	9	445				
	4	395				
	5	398				
Aspergillus sp. 7	6	398				
	7	400	10	Trihydroxamate	Light Brown	Hexadentate
	8	400				
	9	405				
	4	421				
	5	420				
Aspergillus sp. 9	6	421				
	7	425	4	Trihydroxamate	Light Brown	Hexadentate
	8	423				
	9	422				
	4	437				
	5	440				
Aspergillus sp. 10	6	445				
	7	435	15	Trihydroxamate	Dark Brown	Hexadentate
	8	433				
	9	430				
	4	400				
	5	398				

Table 7. Continues

Penicillium sp. 1	6	402				
	7	398	2	Trihydroxamate	Yellow	Hexadentate
	8	398				
	9	398				
	45	451448				
Penicillium sp. 2	6	445				
	7	450	7	Trihydroxamate	Dark	Hexadentate
	8	443			Brown	
	9	443				
	4	398				
	5	396				
Penicillium sp. 3	6	396				
	7	400	7	Trihydroxamate	Orange	Hexadentate
	8	403				
	9	400				
	4	435				
	5	417				
Fusarium sp. 1	6	422		Trihydroxamate	Dark	Hexadentate
	7	410	25		Brown	
	8	410				
	9	420				
	4	410				
	5	410				
Fusarium sp. 2	6	411				
	7	410	2	Trihydroxamate	Dark	Hexadentate
	8	412			Brown	
	9	410				
	4	410				
	5	410				
Fusarium sp. 3	6	411				
	7	410	2	Trihydroxamate	Dark	Hexadentate
	8	412			Brown	
	9	410				
	4	425				
	5	425				
UN F 1	6	425				
	7	420	5	Trihydroxamate	Brown	Hexadentate
	8	420				
	9	420				
	4	400				
	5	400				
UN F 2	6	399				
	7	400	1	Trihydroxamate	Brown	Hexadentate
	8	400				
	9	400				
	4	410				
	5	410				
UN F 3	6	410				
	7	408	2	Trihydroxamate	Yellow	Hexadentate
	8	409				
	9	408				

need for siderophore production[24]. Certain marine isolates also have been found to replace ferredoxins that require iron to flavodoxins[25]. But total abandonment of iron cannot be denied, as all iron requiring enzymes are an imperative need of life. "Non detection" could perhaps have been the phenomenon rather than non- production.

Chemical characterization and ligand denticity of siderophores revealed trihydroxamate hexadentate siderophores to predominate in the rhizosphere of the four crop plants. The denticity correlated with the hydroxamate units. The trihydroxamate siderophores were invariably hexadentates as each hydroxamate siderophore by virtue of two oxygen atoms in each, forms a bidentate ligand with metal ion[26]. The iron biding affinity of trihydroxamate siderophores is high as compared to bidentate and tetradentate siderophores. Hence, hexadentate siderophores possess a higher stability constant (chelate effect) than their monomeric precursors, indicating a selective advantage in siderophore evolution [27]. There are reports on predominance of trihydroxamate hexadentate siderophores in marine environment[28]. Hence, irrespective of habitat as terrestrial or marine, trihydroxamate hexadentates predominate in nature[29, 30] have similarly reported dominance of trihydroxamate hexadentates in fungi belonging to diverse groups as *Asco-*, *Basidio-*, *Zygomycota* and Mitosporic fungi, followed by dihydroxamates in some *Basidiomycota*. Monohydroxamates could not be detected.

Differences in the quantity of siderophore production are a natural biological phenomenon. Several reports on strain to strain variation in production are available[31]. Maximum siderophore production was by *Fusarium* sp. 3 and 2 from cotton and least by *Aspergillus* sp. 7 also from cotton Thus, irrespective of the dominant *Aspergillus* spp., *Fusarium* sp 3 and 2 can prove to be potential candidates, for plant growth promotion and disease suppression against the plant pathogenic species in the rhizosphere by competitive inhibition of growth by their higher binding affinity for Fe^{3+} through siderophores. The presence dominant microflora in the rhizosphere of crop plants does not seem to have an edge on plant growth promotion. even though a*spergilli* was the predominant flora and the most diverse while *Fusarium* constituted species as just present. Several studies have shown a relationship between increased colonization of the rhizosphere by a non-pathogen associated with disease suppression, this is well established by the observation of non-pathogenic strains of *Fusarium oxysporum* controlling pathogenic *Fusarium oxysporum* on a variety of crop plants[32].

Table 8. Quantification of hydroxamate siderophores (DFOM µg/ml)

Test Isolates	CAS assay OD at 630 nm	Siderophore production (DFOM µg/ml)
Aspergillus sp. 1	0.40	94
Aspergillus sp. 2	0.48	98
Aspergillus sp. 3	0.70	159
Aspergillus sp. 4	0.60	145
Aspergillus sp. 5	0.51	105
Aspergillus sp. 7	0.20	47
Aspergillus sp. 9	0.38	91
Aspergillus sp. 10	0.40	152
Penicillium sp. 1	0.31	68
Penicillium sp. 2	0.85	201
Penicillium sp. 3	0.93	208
Fusarium sp. 1	0.60	139
Fusarium sp. 2	1.48	346
Fusarium sp. 3	2.0	468
UN F 1	0.58	138
UN F 2	0.95	322
UN F 3	0.29	66

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