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Siderophore –The Iron Chelator Production Potential of Bacteria Associated with Diverse Crops and Growth Medium Optimization for its High Production

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A B S T R A C T

Siderophores (in Greek: iron carriers) are low molecular weight compounds produced under iron-limiting conditions by microorganisms that chelates Fe^{3+} (ferric iron) with high specific activity, which in turn make it available to the plant system. Selection of efficient siderophore producing plant associated bacteria and their potential role in enhancing plant iron uptake is a strategic approach for improving plant health as Fe is an integral component and cofactor for many biomolecules. In the present study 154 bacteria associated with maize, mustard and sugarcane as endophytic or rhizospheric isolates, on screening led to the identification of 24 efficient siderophore producers (Sid+) with siderophore producing index (SPI) of 1.03-1.70 and the concentration from 0.1 to 11.25 µg/ml in Fiss glucose medium. For higher siderophore production optimization minimal and complex media were tested. Barbhaiya and Rao medium (BR), a minimal medium improved siderophore production ranging from 02.54 – 15.65 µg/ml. Complex malt extract medium produced 0.27-2.44 µg/ml of siderophores and was found to have least influence on siderophore production irrespective of bacterial culture. Differential sugars utilization pattern of 35 sugars tested was recorded with different isolates. A formulation of such siderophore producing bacterial isolates can be used for improving micronutrients availability for the plant to be more healthy and productive.

Introduction

Iron is the most crucial and highly demanded micronutrient for plant growth. Under limiting condition small addition can have profound impact on photosynthetic productivity and quality of product. The concentrations of ferrous iron in dissolved state is nearly 10^{-10} to 10^{-9} M (Kraemer 2004) but the ferrous iron level required by all organisms including microbes and higher plants are around 10^{-7} to 10^{-5} M (Poole and McKay, 2003). Although soil iron content far exceeds the plant requirement, its availability especially in calcareous and alkaline soil (pH>7.5) is limited as Fe^{2+} is readily oxidized to Fe^{3+} forming oxyhydroxide polymers of sparing solubility (Radzki *et al.,* 2013) and the estimated area of alkaline soil in India is 3788159 ha (CSSRI, 2015) occupying large

portion of Indo-Gangetic plain. To curb this iron deficiency induced chlorosis normally foliar spraying of $FeSO_4$ is recommended. The imbalanced leaf penetrations, decreased translocation efficiency and scorching effect restrict the foliar application in many crops. The chelators such as Fe-EDTA, EDDHA are also applied in soil widely for mitigating iron deficiency but the increasing awareness towards the impacts of chemicals in soil and water contamination compels to find alternate efficient organic iron chelator. Siderophores are low molecular weight (<500 dalton), mostly water soluble chemically diverse compounds that have high for Fe^{3+} ($>10^{30}$) respectively. The term coined by Lankford (1973) which in Greek means "iron carrier" are high affinity Fe^{3+}chelating compounds with formation constants approximately from 10^{20} to 10^{50} (Ahmed and Holmstrom 2014). The value of insights provided in number of studies that have successfully used pyochelin and pyoverdine bacterial siderophores from *Pseudomonas*, the search for efficient siderophore producing bacteria from diverse crops is continuing. Enterochelin is the strongest chelator that have stability constant of 10^{52} M^{-1} significantly greater than EDTA that have10^{25} M^{-1} have been known.

Siderophore can be classified based on the organism that produces (bacteria mostly aerobic and facultative anaerobic, fungi, plant), chemical nature (Catecholate, hydroxamate, carboxylate, mixed derivative, mugineic acid) and cross reactivity (homologous and non-homologous). Fungi mostly produce hydroxamate type while bacteria produces non-specific and plant produces always mugineic acid called as phytosiderophores. The microbial siderophore chelates ferric iron in hexadendate octahedral complex and once reductase reduces ferric to ferrous dissociates from the complex leaving free siderophore and ferrous iron. The most important prerequisite for siderophore production is the prevalence of iron deficient condition as the presence of Fe^{2+} binds to Ferric uptake repressor complex and stops the transcription of genes involved in siderophore biosynthesis. The excretion of siderophores by rhizosphere bacteria may help the plants in two ways, either stimulation of plant growth by improving the Fe nutrition of the plant (Crowley *et al.,* 1988) or inhibition of the establishment of plant pathogens.

The universal method to detect siderophores using Chrome Azurolsulfonate (CAS) reagent plate assay, as CAS-HDTMA complex has high affinity towards ferric ion resulting in dark blue colour. When the siderophore is added, the siderophore binds with the ferric iron, releasing free dye leading to orange halo zone colour. Although universal siderophore production protocol which gives high siderophore production from wide range of microbial cultures would be desirable, this goal might be certainly impractical given the negative repressible system of siderophore biosynthesis, low reproducibility of quantified siderophore data. Therefore several options have been developed for optimisation of production in specific microbial systems. As a first step toward this objective, we screened native populations of siderophore-producing bacteria colonizing the rhizo and endosphere of maize (*Zea mays*), mustrad (*Brassica* species) and sugarcane (*Saccharum officinarum*) using CAS assay and used a methodological framework to classify the chemotype and optimize the siderophore production by different microbial isolates.

Materials and Methods

Collection of bacterial isolates

One fifty four putative siderophore producing isolates of which 42 endosphere isolates from maize varieties PEEHM5 and PC4, 33 endosphere isolates from *Brassica juncea*

variety PM25 (Marag *et al.,* 2018) and *Brassica carinata* varieties Pusa Swarnim and Pusa Aditya and 79 rhizospheric isolates from sugarcane variety CoLK94184 were collected for screening of efficient siderophore producing isolates. All isolates were cultured using nutrient agar medium after incubation at 30°C for 24-48 hrs and maintained as glycerol stock at -20^{0}C.

Qualitative screening of bacterial isolates for siderophore production (Sid)

CAS plate preparation

Add 2 ml of 50% glucose solution to 88 ml of basal agar medium (per litre MOPS, 3g; KH_2PO_4, 0.03g; NaCl, 0.05g; NH_3Cl, 0.01g; L-asparagine, 0.05; agar-agar, 18g, pH 6.8-7.0). 10 ml of the CAS indicator solution was added to 90 ml of basal agar-glucose medium with gentle stirring. Once mixed thoroughly, the resulting solution (100 ml) was poured into sterile plastic plates. CAS indicator solution was prepared by adding 40 ml of HDTMA solution (10mM) to 50 ml of chrome azurol S solution (2mM) gently with continuous shaking. 10 ml of FeIII solution was mixed to 90 ml of CAS-HDTMA solution the dark blue colour solution obtained was autoclaved separately at 121^{0}C at 15psi for 20min.

Preparation of bacterial culture suspension and screening using CAS plate assay

Bacterial suspensions of all 154 bacterial isolates were prepared by inoculating each in separate 5ml of nutrient broth, incubated under shake culture at 30^{0}C for 24 hrs at 120 rpm. Bacterial Siderophore producing ability was screened on CAS plates. Each plate was divided in nine sectors and each sector was inoculated with a spot of 10 µl bacterial suspension containing approximately 10^4 bacterial cells and incubated at 30°C for 2-3 days. The deep yellow to orange zone around the colony indicates positive for siderophore production.

Quantitative screening of siderophore producing isolates (Sid$^+$)

Cell free preparation for siderophores

The quantitative analysis for production of siderophore was carried out according to Shelley (1994). Each bacterial culture containing ≈10^6cfu was inoculated in 50 ml broth of Fiss glucose medium (Vellore 2001) and incubated at 30^{0}C for 3 days on rotary shaker (Excella E24 Incubator shaker) at 180rpm. After incubation, supernatant of culture suspension was collected by centrifuging at 14000 rpm for 20 min at 4^{0}C and was used as cell free siderophore preparation.

Estimation of siderophore in cell free preparation by CAS liquid assay

For quantification of siderophores in each preparation, 4 ml of cell free extract was added to 8 ml of CAS solution and incubated in dark for 10 min. The change of colour from blue to yellow was read at optical density 600 nm using Dynamica UV-VIS Double Beam spectrophotometer. The absorbance was converted into concentration basis by using the standard of siderophore desferrioxamine (Sigma).

The quantity of siderophore production was expressed as µg /ml. The standard curve was prepared by taking different concentration from 0 to 50 µM of desferrioxamine and the volume was made to 2 ml. 4 ml of CAS solution were added and incubated in dark for 10 min and the optical density was measured at 600 nm. A standard curve of OD vs. siderophore concentration was plotted.

Effect of growth medium on maximization of siderophore production

Culturing siderophore isolates in different medium

The minimal medium such as Siderophore Inducing Medium (SIM) (Alexander and Zuberer1991), Casamino acid medium (CAAS) (Schwyn and Neilands 1987), Barbhaiya and Rao (1985) medium (BR) and the complex Malt extract medium (ME) were prepared and autoclaved at 121^0C at 15psi for 20minutes. Each bacterial culture containing ≈10^6cfu/ml was inoculated in 50 ml broth of these media and incubated at 30^0C for 3 days on rotary shaker at 180rpm.

Quantification of siderophores in different medium

The cultures were centrifuged at 14000 rpm for 20 min at 4^0C and the quantification of siderophore in cell free extract were determined as mentioned above

Carbohydrate utilization pattern

The carbohydrate utilisation pattern for selected isolates was carried out using KB009 Hicarbo^{TM} kit based on the principle of redox dye colour change due to substrate utilization. This kit has combination of 35 test sugars and 1 control. The kit contains Part A (KB009A) having 12 sugars, Part B (KB009B) having 12 sugars and Part C (KB009C) containing 11 sugars and 1 control. The pure cultures of the selected isolates were cultured in 5 ml of nutrient broth and incubated at 37°C for 24 hrs until the turbidity measurement at OD620nm reaches ≥ 0.5. The kit was opened aseptically in the laminar air flow and sealing foil was peeled off. 50 µl of prepared bacterial isolates were inoculated on the surface of each well in the plates. The plates were incubated at 37°C for 24 hrs and sugar utilization pattern results were recorded by the interpretation chart available with the kit.

Results and Discussion

Efficient siderophore producing bacteria

Different crops associated 154 bacterial isolates on screening for siderophore production capability using CAS plate assay (Fig. 1) led to selection of 64 siderophore positive (Sid+) isolates, which included 8 isolates from 42 maize isolates, 19 from 33 mustard isolates and 37 from 79 sugarcane isolates (Table 1). These isolates exhibited variable siderophore production potential as index for siderophore production (SPI) varied from 1.03 to 1.70 (Table 2). Quantification of siderophore produced in a defined Fiss glucose (FG) medium led to the identification of 24 efficient siderophore producers and the range of siderophore production among different isolates in Fiss glucose medium varied from 0.1 to 11.25 µg/ml. Three isolates from sugarcane did not show any quantitative siderophore production. Siderophore production in Fiss Glucose Minimal medium depends on bacterial isolates potential and conditions but favourably a biasness seen towards the maize endophytic culture producing large amount of siderophores (Fig. 2).

Growth medium vs. siderophore production potential

For maximizing siderophore production, different minimal and complex media were used (Table 3). Complex malt extract medium produced 0.27-2.44 µg/ml of siderophores and was found to have least influence on siderophore production irrespective of bacterial culture implicating the difficulty in maintaining iron deficient condition in complex medium.

Table.1 Qualitative screening of isolates from different crops for siderophore production

Sr No.	Crop	Isolation Source	Isolates	Sid+ve isolates	Efficient Sid+ isolates
1	*Zea mays*	Endosphere	42	08	08
2	*Brassica sp*	Phyllo and endosphere	33	19	04
3	*Saccharum officinarum*	Rhizo and Endosphere	79	37	12
		Total	154	64	24

Table.2 Siderophore production indices of Sid+ve isolates

Sr No	Crop	Variety	Source	Isolate No.	Bacterial colony (mm)	Siderophore zone (mm)	SPI	Efficient Sid+
1	*Zea mays*	PEEHM5	Endosphere	PHM22	17.5	2.5	1.14	Sid1
2	*Zea mays*	PEEHM5	-do-	PHM23	18.9	2.1	1.11	Sid2
3	*Zea mays*	PEEHM5	-do-	PHM30	17.3	1.3	1.08	Sid3
	Zea mays	PEEHM5	-do-	PHM37	16.5	2.8	1.17	Sid4
4	*Zea mays*	PEEHM5	-do-	PHM59	18.7	1	1.05	Sid5
5	*Zea mays*	PEEHM5	-do-	PHM67	20.5	1.5	1.07	Sid6
6	*Zea mays*	PC4	-do-	PC31	17.7	2.13	1.12	Sid7
7	*Zea mays*	PC4	-do-	PC42	17.5	1.4	1.08	Sid8
8	*Brassica juncea*	PM25	Phyllosphere	PM20	9.5	1.5	1.16	Sid9
9	*Brassica juncea*	PM25	-do-	PM2	11.5	0.5	1.04	
10	*Brassica juncea*	PM25	-do-	PM3	8.5	0.5	1.06	
11	*Brassica juncea*	PM27	Endosphere	PM7	10.4	0.6	1.06	
12	*Brassica juncea*	PM27	-do-	PM8	8.5	1.5	1.18	
13	*Brassica juncea*	PM27	-do-	PM9	7.6	1.4	1.18	
14	*Brassica juncea*	PM27	-do-	PM10	9.8	0.2	1.02	
15	*Brassica juncea*	PM30	-do-	PM11	11.5	0.5	1.04	
16	*Brassica juncea*	PM30	-do-	PM12	12.1	0.9	1.07	
17	*Brassica juncea*	PM30	-do-	PM17	13.2	0.8	1.06	
18	*Brassica juncea*	PMVijay	Phyllosphere	PM18	11.5	1.5	1.13	
19	*Brassica juncea*	PMVijay	-do-	PM21	18.9	1.1	1.06	
20	*Brassica carrinata*	Aditaya	-do-	PMAL4	17.8	1.2	1.07	Sid10
21	*Brassica carrinata*	Aditaya	-do-	PM34	22.7	1.3	1.06	Sid11
22	*Brassica carrinata*	Swarnim	-do-	PM37	18.5	1.5	1.08	Sid12
23	*Brassica carrinata*	Swarnim	-do-	PM42	13.5	3.5	1.26	
24	*Brassica carrinata*	Swarnim	-do-	PM43	14.5	1.5	1.10	
25	*Brassica carrinata*	Swarnim	-do-	PM44	13.7	0.3	1.02	

26	*Brassica carrinata*	Swarnim	-do-	PM46	11.9	1.1	1.09	
27	*Brassica carrinata*	Swarnim	-do-	PM47	14.7	2.3	1.16	
28	*Saccharum officinarum*	CoLK94184	Rhizosphere	SR1	16.5	3.3	1.20	Sid13
29	*Saccharum officinarum*	CoLK94184	-do-	SR3	10.9	2.6	1.24	
30	*Saccharum officinarum*	CoLK94184	-do-	SR4	19.6	3.9	1.20	Sid14
31	*Saccharum officinarum*	CoLK94184	-do-	SR5	15.6	6.4	1.41	Sid15
32	*Saccharum officinarum*	CoLK94184	-do-	SR6	20.6	0.9	1.04	Sid16
33	*Saccharum officinarum*	CoLK94184	-do-	SR7	19.7	3.3	1.17	Sid17
34	*Saccharum officinarum*	CoLK94184	-do-	SR10	16.7	1.9	1.11	Sid18
35	*Saccharum officinarum*	CoLK94184	Rhizoplane	SP1	10.5	1.5	1.14	
36	*Saccharum officinarum*	CoLK94184	-do-	SP3	13.7	1.3	1.09	
37	*Saccharum officinarum*	CoLK94184	-do-	SP4	12.9	1.1	1.09	
38	*Saccharum officinarum*	CoLK94184	-do-	SP6	15.5	0.5	1.03	
39	*Saccharum officinarum*	CoLK94184	-do-	SP7	16.2	0.8	1.05	
40	*Saccharum officinarum*	CoLK94184	-do-	SP9	10.6	1.4	1.13	
41	*Saccharum officinarum*	CoLK94184	-do-	SP13	11.9	1.1	1.09	
42	*Saccharum officinarum*	CoLK94184	-do-	SP14	13.5	0.5	1.04	
43	*Saccharum officinarum*	CoLK94184	-do-	SP17	12.8	3.2	1.25	
44	*Saccharum officinarum*	CoLK94184	-do-	SP18	12.9	2.1	1.16	
45	*Saccharum officinarum*	CoLK94184	-do-	SP19	13.4	2.6	1.19	
46	*Saccharum officinarum*	CoLK94184	-do-	SP22	12.8	4.2	1.33	
47	*Saccharum officinarum*	CoLK94184	-do-	SP25	20.9	7.1	1.34	Sid19
48	*Saccharum officinarum*	CoLK94184	-do-	SE27	12.3	3.7	1.30	
49	*Saccharum officinarum*	CoLK94184	-do-	SE31	16.7	1.3	1.08	Sid20
50	*Saccharum officinarum*	CoLK94184	-do-	SE34	15.3	1.7	1.11	
51	*Saccharum officinarum*	CoLK94184	-do-	SE36	13.8	2.2	1.16	
52	*Saccharum officinarum*	CoLK94184	-do-	SE39	15.6	1.4	1.09	
53	*Saccharum officinarum*	CoLK94184	Endophyte	SE41	16.7	2.8	1.17	Sid21
54	*Saccharum officinarum*	CoLK94184	-do-	SE42	14.5	1.5	1.10	
55	*Saccharum officinarum*	CoLK94184	-do-	SE51	12.3	1.7	1.14	
56	*Saccharum officinarum*	CoLK94184	-do-	SE53	13.5	1.5	1.11	
57	*Saccharum officinarum*	CoLK94184	-do-	SE60	12.9	4.1	1.32	
58	*Saccharum officinarum*	CoLK94184	-do-	SE63	11.9	4.1	1.34	
59	*Saccharum officinarum*	CoLK94184	-do-	SE67	15.6	2.4	1.15	Sid22
60	*Saccharum officinarum*	CoLK94184	-do-	SE76	11.2	2.8	1.25	
61	*Saccharum officinarum*	CoLK94184	-do-	SE82	10.5	7.4	1.70	Sid23
62	*Saccharum officinarum*	CoLK94184	-do-	SE88	12.6	3.4	1.27	
63	*Saccharum officinarum*	CoLK94184	-do-	SE91	13.6	4.2	1.31	Sid24
64	*Saccharum officinarum*	CoLK94184	-do-	SE105	21.3	0.8	1.04	

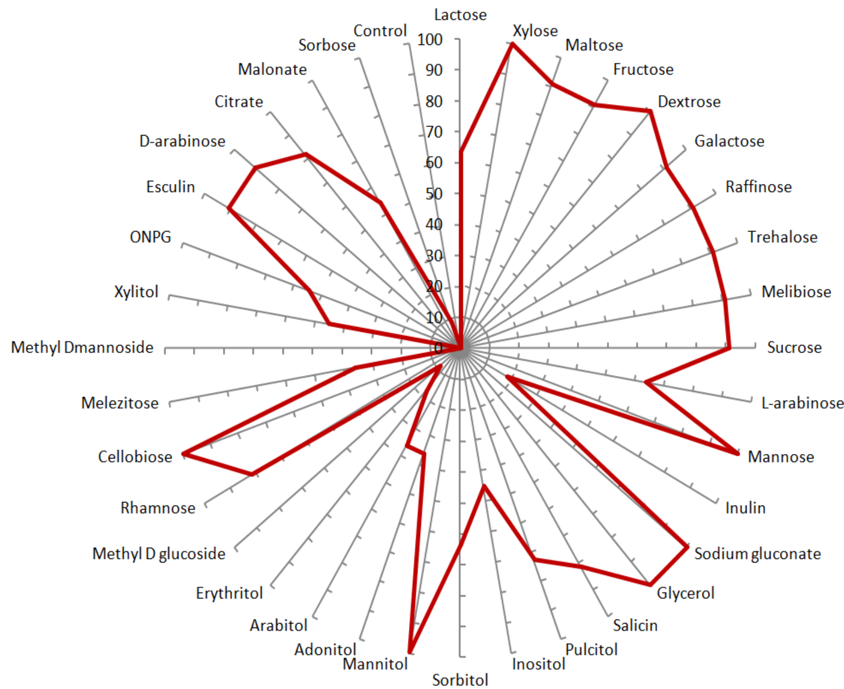
Table.3 Quantification of siderophore production in different growth media

Bacterial Isolates	Siderophore (µg/ml) in different growth medium				
	FG	**BRB**	**SIM**	**ME**	**CAAS**
Sid01	2.58	4.56	0.4	0.26	5.33
Sid02	5.68	5.67	7.02	0.4	8.15
Sid03	4.13	3.65	1.95	0.13	6.67
Sid04	7.58	12.76	0.54	0.89	9.84
Sid05	3.01	5.78	2.44	0.35	5.68
Sid06	8.99	7.86	8.43	0.68	11.11
Sid07	11.25	3.43	2.02	0.97	13.5
Sid08	0.4	2.54	0.23	1.25	3.29
Sid09	6.7	5.64	1.9	1.74	5.19
Sid10	2.51	9.87	0.44	0.75	3.08
Sid11	3.1	4.56	5.12	0.96	5.26
Sid12	0.1	3.45	10.12	2.44	12.65
Sid 13	0.05	2.64	3.23	1.56	5.23
Sid14	2.5	5.64	3.53	1.88	11.11
Sid15	10.68	6.78	11	1.46	3.85
Sid16	0.57	1.65	0.65	1.23	1.25
Sid17	8.9	11.22	8.34	0.75	3.99
Sid18	0.37	14.56	1.53	0.47	4.91
Sid19	4.32	18.76	0.33	0.82	5.82
Sid20	1.15	4.24	0.54	1.23	3.65
Sid21	0.96	19.65	0.68	0.33	7.73
Sid22	0.3	14.56	2.58	0.75	3.78
Sid23	8.92	12.34	0	0.4	3.5
Sid24	2.09	10.98	2.44	0.96	5.68
Sem±	2.65	1.59	0.42	0.35	1.48
CD(P=0.01)	0.84	4.5	1.2	1.01	4.21

FG-Fiss Glucose Medium, BRB- Barbhaiya and Rao medium, SIM- Siderophore Inducing medium, ME- Malt extract medium, CAAS- Casamino acid medium

Fig.1 Holozones produced by the Sid+ bacterial isolates

Fig.2 Sugar utilisation frequency of different Sid+ isolates



Iron is the essential micronutrient required by all the living organisms. It is involved in various metabolic processes such as respiration, photosynthesis and nitrogen fixation. Normally foliar spray of ferrous sulphate and chemical chelators were recommended to mitigate iron deficiency. The increased awareness towards the impact of chemical fertilizers in soil leading to water and soil contamination and recalcitrant nature of these chemical chelators for long period compels to find alternate organic iron chelators like siderophores. In the present study 154 bacterial isolates from maize, mustard and sugarcane were screened and 24 efficient siderophore producing isolates were selected. Jenifer *et al.,* (2015) reported siderophore producing bacteria from aquatic environment, wherein, from a population of 125 isolates only 12 morphologically different siderophore producing isolates were identified. Storey (2005) working with *Rhizobium leguminosarum* has indicated that under normal condition using Fiss glucose medium 0.2mg/ml of hydroxamate siderophore was produced which can further be optimized to nearly 0.72 mg/ml with certain modifications in the growth medium. Here modulation of growth conditions mainly medium composition influenced siderophore production and with BR medium siderophore production improved to 02.54 – 15.65 μg/ml. This clearly states that growth medium has a profound effect on secondary metabolite production. There are several reports for varied growth media for enhanced siderophore production like succinate medium, casamino acid medium and malt extract medium (Mayer and Abdullah 1978; Ali and Vidhale 2013). The other culturable conditions have also been tried for the enhanced production of siderophores as Katiyar and Goel (2004) selected low temperature tolerant fluorescent pseudomonads which could grow at both mesophilic and psychrophilic temperature and

produced 17 fold enhanced siderophore production. Pratty *et al.,* (2008) selected mutant strains of soil bacterium *Rhodococcus erythropolis* for overproduction of siderophore which shows two fold enhanced activity than its wild strain.

Chemically siderophore produced by different organisms have been reported of diverse nature based on organism type, chemical nature and their cross-reactivity. Ali and Vidhale (2013) reported that nearly 500 different siderophores have been identified, (http://bertrandsamuel.free.fr/siderophore_base/siderophores.php) while 270 have been chemically characterised. The remaining siderophores have to be explored in the future and the roles of these siderophores in the environment are yet to be discovered. Chemically they belong mainly to catecholate, hydroxamate, carboxylate and mixed ligands type (Ratul *et al.,* 2013). The colonizing population of a plant in rhizosphere and endosphere may produce either one or mixed type of siderophores for efficient uptake of available iron. Similar results have been reported by Jenifer *et al.,* (2015) that *E. coli* strains were producing hydroxamate and *P. aeruginosa* producing catecholate nature siderophores. Zawadzka *et al.,* (2006) identified siderophores of *P.stutzeri* and have explained out of 12 isolates, 6 strains produced proferrioxamine the hydroxamate siderophore, 3 produced amonabactin the catecholate type siderophores. In this study, out of 24 isolates 9 isolates produced catecholate, 5 isolates produced hydroxamate and 1 produced carboxylate type of siderophore. Identified four isolates from mustard produced both catecholate and hydroxamate type of siderophores whereas three identified isolates each from maize and sugarcane were catecholate and hydroxamate respectively. Paul and Dubey (2015) have reported that *Rhizobium sp.* produced catecholate and hydroxamate siderophore, *Bacillus sp.* produced hydroxamate and yeast produced catecholate. Though iron is not provided in the medium (in BR medium no iron added), siderophore production is induced as little amount of iron will mostly associated as impurity in medium components providing an iron deficient condition. The particular iron concentration for particular bacterial isolate optimization is important as increase can be observed without addition of iron and correct iron concentration maintenance.

Understanding that the plant recruits its microbiome has received substantial attention in recent years (Dennis *et al.,* 2010). It is done mainly through its rhizo-depositions in the soil as root exudates, sloughing of root cells and the release of mucilage deposits. Root exudates, containing a variety of compounds, predominately organic acids and sugars, but also amino acids, fatty acids, vitamins, growth factors, hormones and antimicrobial compounds, becomes key determinants of rhizosphere microbiome structure. The composition of root exudates can vary between plant species and cultivars, and with plant age and developmental stage (Bertin *et al.,* 2003). All Sid+ isolates were screened for carbohydrate utilisation profiling and 16S rRNA gene profiling Majority of isolates were able to utilize more than 20 out of 35 tested sugars clearly indicated their versatility of metabolic activities to sustain life and beneficial colonization with the plant. The sugars like xylose, dextrose, mannose, sodium gluconate, glycerol, mannitol and cellobiose were easily metabolized as carbon source whereas sugars like methyl D gluconate was not acceptable by majority of bacteria. The maximum number of sugars was utilised by the isolate Sid11 as it utilizes 31 sugars of 35 tested sugars.

Overall this study clearly indicates that different crops recruit variable microflora and

this selection is based on mutual positive interactions. Generally the colonizing microbial population exhibit several plant growth promoting characters. Increasing the availability of Fe for the plant through siderophores is a very notable character. Isolates selected in the study will be used in developing commercial siderophore preparation.

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