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# ISOLATION AND CHARACTERIZATION OF SIDEROPHORE PRODUCING ENDOPHYTIC BACTERIA FROM LANDRACE RICE VARIETY OF JHARKHAND

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Siderophore production by microbes is an important mechanism for plant growth promotion and disease suppression. In this study four land races of different rice Sada Gora(SG), Bala Gora (BG), Kala Gora(KG) and Kharani(KH) with several of ethnomedicinal properties were taken for the study. 13 endophytes(BGS2 ,BGS3, BGR7, BGR10, BGR12, KHS1 KHR1,KHR2, SGS1 ,SGS2, SGS3,KGR2,KGS1) were isolated from stem and root of the selected variety of rice and screened for Siderophore production (CAS assay ). 10 isolates BGS2, BGS3, KHS1, KHR1, SGS1, SGS2, SGS3, KGR2, KGS1 gave +ve test for siderophore production .Theses endophytes were characterised at molecular level(16 S rDNA) and the strains identified were *Pantoea sp.* BSJ2*, Pantoea stewartii, Pseudomonas sp., Sphingomonas sp.* LK11, *Pantoea ananatis, Burkholderia cepacia, Xanthomonas sacchari, Pseudomonas sp., Pantoea sp., and Agrobacterium tumefaciens.*

**Key Words:** *Diazotrophic, Endophytic Bacteria, Ethenomedicinal, Siderophore.*

Siderophore is one of the traits that makes microorganisms successful competitors in various environments and facilitate plant bacterial association as well as colonization of roots, stem and leaf. Thus, make iron unavailable to pathogenic microorganism (Chincholkar *et al.* 2005). Siderophores are extracellular, small (low molecular weight < 1000 Daltons) compounds, which selectively bind iron (Fe3+). The siderophores are generally produced by microorganisms, both aerobic and facultative anaerobic and monocotyledonous plants under low-iron stress conditions (Seuket *et al.* 1998). The Iron is required in large abundance, as it is involved in various important biological processes, such as photosynthesis, respiration, chlorophyll biosynthesis and biological nitrogen fixation, for plant growth promotion (Ahemad and Khan 2012)and disease suppression (Arruda et al. 2013). Siderophore producing bacteria have been used as biocontrol agents to combat plant pathogens (Neilands 1974). Iron plays a central role in the energy metabolism of aerobic and semi-aerobic microorganisms (Eland and Baker 1985). Its availability in soil form microorganisms and plants drops dramatically with increasing pH above 6. The first report of a siderophore production was reported from *Ustilago sphaerogena* (Gram 1996). Typically, microbial sideophores are classified as catecholares, hydroxamates and α-carboxylates, depending on chemical nature of their coordination sites with iron (Kumar *et al.* 2013). Rice genotypes differ widely in their tolerance to iron toxicity (Barraquio *et al.* 2000). Siderophore produced by majority of PGPR (Benson 1998). including rhizobia has been suggested as one of the modes of growth promotion of nodulated legumes under field conditions where in siderophores facilitate the uptake of iron (assimilation) from the environment (Xalxo 2008). A nodulated legume has been found to have an increased demand for iron compared to that of a non-nodulated plant (Singh *et al*. 2010). With respect to plant health, the presence of genes for siderophore production indicates strong biocontrol capacities allowing competition with pathogens for iron, as suspected for some cultured endophytes (G Kaur 2016) reported that genus *Bacillus* and *Enterobacter* have ability to produce siderophore, also improve rhizosphere colonization (Etesami *et al.* 2013).

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documented that bacterial genera *Pseudomonas*, *Burkholderia* and many species of *Pantoea*in rice have antagonistic activity owing to siderophore production. As reported *P. fluorescens* NCIM 5096 and *P. putida* NCIM 2487 were able to produce siderophores (Hung *et al.* 2007). Cultivation of indigenous variety has become marginal because of intervention of hybrid and HYV. Change in cultivation pattern due to mining and application of heavy fertilizers in hybrid and HYV (Xalxo P 2008) in rice production has resulted in huge genetic erosion of indigenous rice gene pool and soil nutrient disturbance in the states. In order to make indigenous rice cultivation in Jharkhand sustainable, it is extremely important to combine new microbial technology with traditional knowledge in the area. Recently, microbioal siderophores are isolated, purified and utilized, in agriculture field in cancer therapy, as antimalaria, andisleeping sickness (Wandersman and Delepelaire 2008).

Bacterial endophytes can offer several benefits to the host plant, particularly growth promotion, protection from pathogens and under diverse environmental conditions.The main objective of this study were to isolate endophytic diazotropic bacteria from indigenous rice having ethnomedicinal property and screen them for siderophore production and optimise the time and temperature of its production. Analysis of the type of siderophore production was also done.

## MATERIAL AND METHOD

**Sources of Endophytic Diazotropic Bacteria for siderophore Production:** Indigenous rice varieties Sada Gora, Bala Gora, Kala Gora and Kharani were chosen for isolation of endophytic diazotrophic bacteria. The selection of these rice varieties for isolation of endophytic bacteria was based on the level of available nitrogen in three particular fields from where plants were collected. The Endophytic bacteria were isolated from indigenous rice grown by the indigenous people of Thakurgaon Village of Ranchi District, Jharkhand and, important features of the selected varieties and their cultivation pattern are given in the.

Endophytic bacteria were isolated from different parts of selected rice plants such as root and stem of selected plants employing standard microbiological methods. Method of (Barraquio *et al.* 1997) was used for isolation. The suspension was diluted to $10^{-2}$ to $10^{-10}$ and 100 µl from each dilution was spread on nitrogen-free JNFb (Jhonsons nitrogen free

**Table-1:** Detail of landrace variety used for Isolation of putative endophytic diazotropic bacteria for siderophore production at Thakurgaon Village of Ranchi District, Jharkhand and important features of selected variety

| Place | Location of the rice field | Latitude and longitude | Rice variety | Land type | Importance |
|---|---|---|---|---|---|
| State- Jharakand Country- India | Village- Thakur Goan District- Ranchi | $23.52^{oN}$ $85.17^{0E}$ at an average elevation of 625m | Sada Gora, Kala Gora, Bala Gora, Kharani | Grown in Tarn1, (Upland) Doin II and Doin III (different depths of low land) as locally called by the tribal farmers. All the varieties are upland variety but can be grown in low land also and the varieties selected are mostly sown by broadcasting method . When planted in low land than transplanting (dhanropany) method, | Oldest known rice which can be stored up to 50 years without the use of any other preservatives Used in the preparation of tribal drink Hadia which is used to cure jaundice. The hadia prepared from Kharani is known as medicinal Hadia |

media).

**Test for Siderophore Production:** The isolates were tested for siderophore production ability following the method of (Neilands 1974). Spot inoculation of test culture was made on CAS-agar plates and incubated for 72 h at $28^0$C. Yellow to orange halo zone appearing around the colonies was recorded as positive test for siderophore production.

**Analysis of Siderophore:** Type of siderophore was determined by method of (Arnow 1937). Bacterial isolates were grown in $JNFb^-$ medium supplemented with 1 µM $FeCl_3$. 1 ml each culture was harvested at 8000 rpm for 5 min and culture supernatant was saved for analysis
.

**Iron Percolate Assay for the Detection of Hydroxamate type siderophore:** This assay is done under acidic condition for the detection and estimation of Hydroxamate type siderophore by Iron Percolate Assay (Atkin *et al.* 1970). An orange-red color will form, which may vary in intensity depending on the quantity of siderophore produced . Absorbance was measured at 480 nm together with inoculated media mixed with reagent as a blank.

**Time course experiment:** Level of siderophore production during growth phase, siderophore production was measured at different time interval 0 Hours,24,48,72, to 144 hours respectively.

**Morphological And Biochemical Characterisation:** Initial Characterization of siderophore producing endophytic bacterial, all the 10 isolates selected and isolated on the basis of morphological characters were routinely grown in $JNFb^-$ solid agar-agar medium. These all routinely showed good growth even after repeated sub culturing. Once it became evident that all these above isolates are indeed putative diazotrophic endophytic bacteria, it was desirable to characterize them so as to find out both physiological and biochemical diversity.

**Biochemical Characterization:** For biochemical characterisation Gram Staining, Urease, Catalase, Nitrate Reductase, Methyl Red were done following standard laboratory protocols.

**Molecular characterization:** Molecular characterization of isolated endophytic diazotrophic siderophore producing bacteria was done in following steps:-

a. Genomic DNA Isolation
b. Isolation was done using DNA isolation kit.

**Identification on the basis of 16S rDNA Nucleotide Sequence:** 1.5 kb rDNA was amplified using universal primer. Amplification of rDNA from pure cultures was performed in a final volume of 50 µl. The PCR reaction mix included 1.5 U of *Taq* DNA polymerase (Bangalore Genei, India), 1 X PCR buffer with 1.5 mM $MgCl_2$, 300 ng each forward and reverse primers (Integrated DNA Technologies, Inc., IA, USA), each dNTP at 125 µM (Bangalore Genei, India) and 50 ng template DNA. Pair of primers used for amplification of rDNA were 8f 5'-AGA GTT TGA TYM TGG CTC AG-3'and 1495r 5'-CTA CGG CTA CCT TGT TAC GA-3' where Y = C or T and M = A or C. Thermal cycles for the amplification were as follows; 4 min at $94^o$ C, 35 cycle of 1 min at $94^o$ C, 1 min at $58^o$ C and 2 min at $72^o$ C followed by 5 min at $72^o$ C and storage at $4^o$C.

16S rDNA sequences were matched against nucleotide sequences present in Gene Bank using the BLAST nucleotide program[173] at website www.ncbi.nlm.nih.gov/blast.

**Confirmation Of Diazotrophy Of The**

**Table-2:** Isolation of putative endophytic diazotrophic bacteria from different parts of selected indigenous rice variety

| Plant/Variety | Part/Organ | Designated Isolates | Total No. of Isolates |
|---|---|---|---|
| Balagora | Root<br>Stem | BGS2, BGS3, BGS6<br>BGR7, BGR5, BGR10, BGR12 | 3<br>4 |
| Sada gora | Root<br>Stem | ---<br>SGS1, SGS2, SGS3 | 0<br>3 |
| Kala gora | Root<br>Stem | KGR2<br>KGS2 | 1<br>1 |
| Karhaini | Root<br>Stem | KHR1,KHR2<br>KHS1 | 2<br>1 |

**Endophytic Isolates**

**DNA SEQUENCING**

Amplified desired genes (*nifH* and 16S r DNA) was sent to Sai biotech for sequencing .the sequence obtained were compared with nucleotide sequence present in gene bank using BLASTn and/or BLASTx program of NCBI.

## RESULTS

**Isolation of putative diazo trophic endophytes from different plants:** Indigenous rice varieties Sada Gora, Bala Gora, Kala Gora and Kharani were chosen for isolation of endophytic diazotrophic bacteria. The selection of these rice varieties for isolation of endophytic bacteria was based on the level of available nitrogen in three particular field from where plants were collected. Different parts of plant such as roots, culm and seed were used for the screening of endophytic bacteria .When macerate of different dilutions of surface sterilized plant's parts such as roots, culm were plated on solid agar-agar NA or JNFb$^-$ medium, a number of discrete colonies of bacteria appeared after 3-4 days of growth. The appearance of bacterial colonies was observed in the macerate of all the parts of plants such as roots and culm. The highest number of bacterial isolates based on differences in morphological difference was observed in the selected rice variety (Table 2 fig 1).

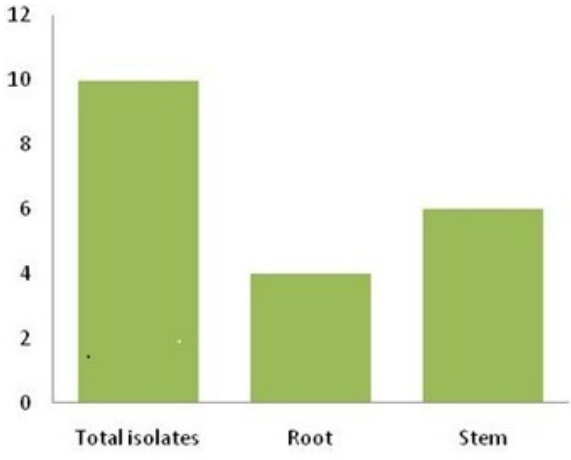
In our preliminary screening, CAS-agar assay (based mainly on the appearance of yellowish halo zone) revealed varying level of siderophore production by different isolates employed in the present study. This observation led us to characterize and quantify siderophore in all these eight isolates. Initial characterization employing standard analytical methods reflected the production of catecholate type of siderophores in all the isolates. Identity of catecholate type of siderophores was solely based on the method of Arnow 1(937).

**Morphological And Biochemical Characterisation:** On the basis of morphological studies **(Table),** endophytic bacteria on nutrient agar medium produced medium sized, irregular shaped, off-white and rough colonies and were tentatively placed in genus *Bacillus* sp. whereas seven isolates produced round shaped and raised colonies with entire margins, mucoid and cream in colour were tentatively placed as *Klebsiella sp.* (2 isolates). Two isolates produced medium sized, round shaped and flat colonies with smooth margin and light yellow to off white colour and tentatively belonged to genera *Pseudomonas* sp. With a view to characterize the bacterial isolates, all the isolates were subjected to a few important biochemical tests. It is evident from the data of **Table 5** that all the isolates were gram negative and showed positive test for catalase (Table 5).

All the isolates were grown on JNFb$^-$ solid agar-agar medium under identical condition.

Colony counting was made after 3 days of growth.

Results are based on average of three replicates.



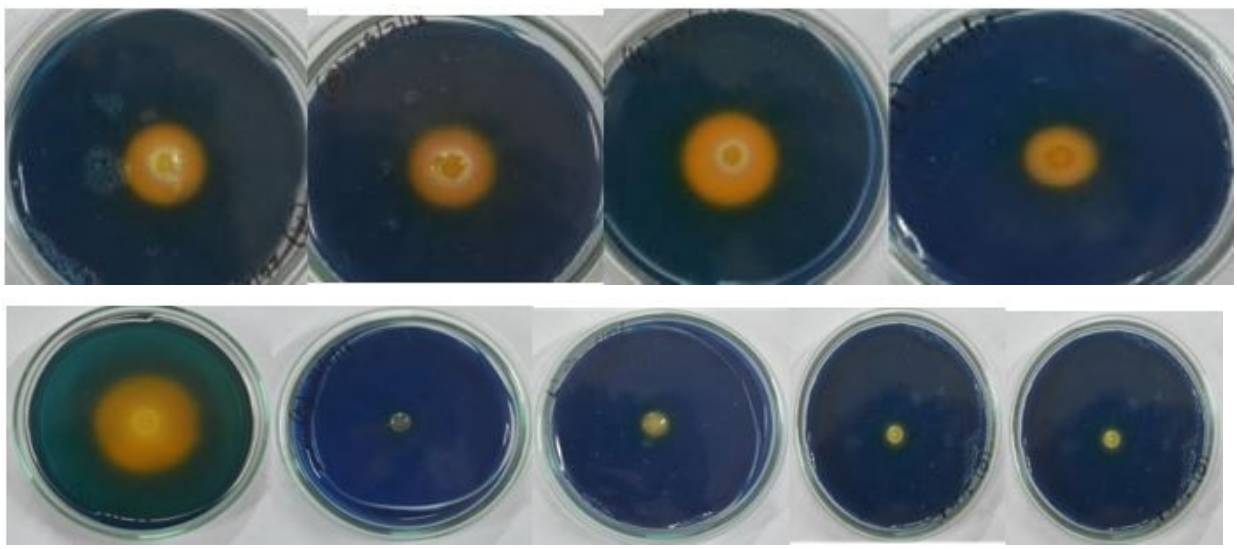

**Graph 1-**Comparative graph siderophore producing isolates from root and stem

All the isolates were grown in DYSG medium except for the test of nitrate reductase and urease for which cultures were grown in JNFb media supplemented with $KNO_3$ or urea.

**Confirmation Of Diazotrophy Of The Endophytic Isolates:** There was an active growth of all the 13 isolates when grown on nitrogen deficient JNFb$^-$ media without supplement of any organic or inorganic sources exogenously. Repeated sub culturing of all the isolates maintained their diazotrophy nature. This reflected the diazotrophic behaviour of all the isolates at the primary level For the molecular analysis of nitrogen fixation, *nifH*gene, one of the structural genes of *nif* operon was amplified (fig 2). Confirmation was made by *nifH*analysis. To achieve this, the presence of ~396 bp fragment of *nifH* in all the10 isolates was tested using universal primers in PCR assay. The genomic DNA of all the 10 isolates showed amplification of ~396 bp fragment of *nifH* (Fig2). However, stringency for the amplification of *nifH* varied in isolates SGS2 and KHS1. *nif H* of SGS2 and KHS1 was amplified at lower stringency (annealing temperature- 52$^o$c) where, there was presence of additional band of ~1100bp in SGS2. If annealing temperature was increased to 55$^o$C, amplification of ~396 bp fragment did not occur in both the above isolates.

## DISCUSSION

The production of siderophore offers a mechanism of plant growth promotion by chelation of $Fe^{+3}$ Siderophore production was detected by means of CAS agar plate assay where blue colour of the medium changed to

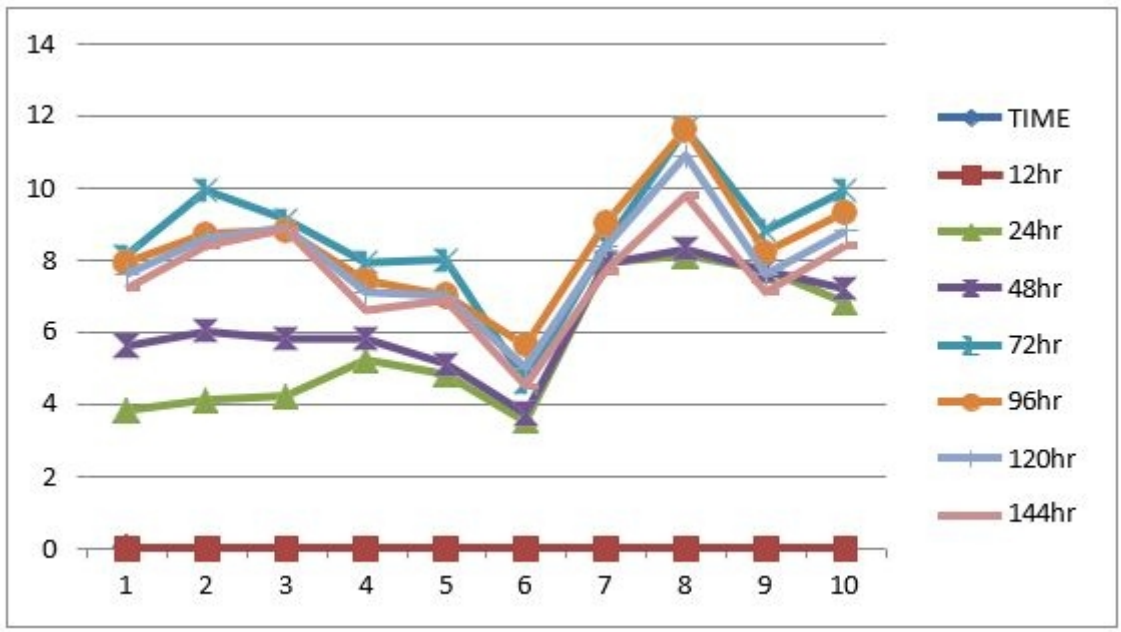


**(Fig:1H,I,J,L,M)**

**Figure 1:**Siderophore production test in selected isolate by plateassay.:siderophore production by individual isolates in the absence of iron.appearance of yellow halo zone appeared in culture growing in Fe deficient
**(A:**BGS2, B:BGS3, C:BGS6, F:BGR10, H:KHS1, I:KHR1, J:KHR2, L:SGS2, M:SGS3)

**Table 3: Time Course Production OF Siderophore** knowing the level of iron in the medium effects the siderophore production considerably, to its production at different phases of growth was desirable

| TIME | 12hr | 24hr | 48hr | 72hr | 96hr | 120hr | 144hr |
|---|---|---|---|---|---|---|---|
| PRODUCT | 0 | 3.8 | 5.6 | 8.1 | **7.9** | **7.6** | **7.2** |
| | 0 | 4.1 | 6 | 9.9 | 8.7 | 8.6 | 8.4 |
| | 0 | 4.2 | 5.8 | 9.1 | 8.8 | 8.89 | 8.85 |
| | 0 | 5.2 | 5.8 | 7.9 | 7.4 | 7.1 | 6.6 |
| | 0 | 4.8 | 5.1 | 7.98 | 6.99 | 6.99 | 6.87 |
| | 0 | 3.5 | 3.7 | 4.6 | 5.6 | 5 | 4.5 |
| | 0 | 7.9 | 7.9 | 8.4 | 8.99 | 8.36 | 7.7 |
| | 0 | 8.1 | 8.3 | 11.6 | 11.6 | 10.9 | 9.8 |
| | 0 | 7.7 | 7.7 | 8.8 | 8.2 | 7.6 | 7.1 |
| | 0 | 6.8 | 7.2 | 9.9 | 9.3 | 8.8 | 8.4 |




**Graph 2:** Time Course Production of Siderophore

**Table – 4:** Morphological characteristics of Endophytic Bacterial Isolates

| Rice Characteristics | Morphology of Endophytic Bacterialisolates | | |
|---|---|---|---|
| Cell Shape | Rods | Rods | Rods |
| Colony Shape | Round | Round | Polymorphic |
| Elevation | Flat | Raised | Umbonate |
| Texture | Mucoid | Mucoid | Mucoid |
| Margin | Smooth | Entire | Irregular |
| Colour | Light Yellow | Cream | Cream |
| Size | Medium | Small | Medium |
| Bacterial Isolates (Tentatively) | *Pseudomonas. Sp.* | *Klebsiella Sp.* | Bacillus Sp. |

· All the isolates were grown on JNFb solid agar-agar medium under identical condition.
· Colony counting was made after 3 days of growth.
· Results are based on average of three replicates

**Table -5 :** Biochemical Characterisation

| Bacterial Isolates | Grams Staining | Urease | Catalase | Nitrate Reductase | Methyl Red |
|---|---|---|---|---|---|
| A | - | - | + | + | + |
| B | - | - | + | + | + |
| F | - | + | + | + | + |
| G | - | - | + | + | _ |
| H | - | - | + | + | _ |
| I | - | + | + | + | + |
| J | - | - | + | + | _ |
| K | - | + | + | + | _ |
| L | - | + | + | + | + |
| M | + | + | + | + | + |

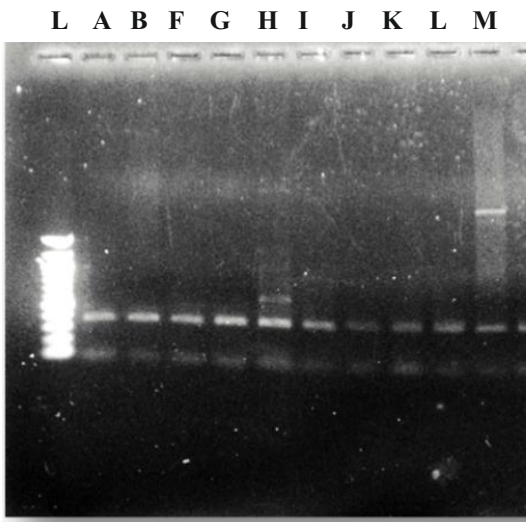



**Figure 2:** Amplification of nifH gene in different isolates. L represents 100bp Ladder and lanes.Lanes A-M corresponds to the 10 endophytic diazotrophic bacterial isolates. The amplification was done with 19F and 107R primer and –veE.Coli JM109.

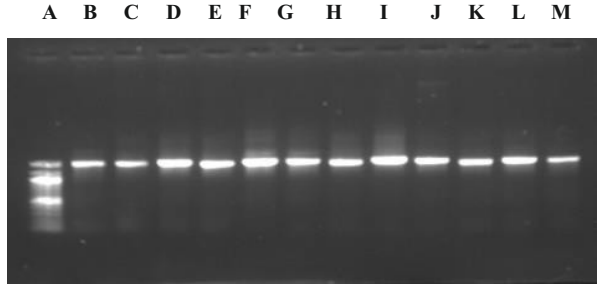



**Figure 3:** Amplified 16S ribosomal DNA from different isolates

yellow/orange around the growing the growing colonies (fig1). The Chromeazurol-s, Iron and CTAB forms a ternary complex, which give a strong blue colour. In limitation of iron in medium, siderophore, a strong chelator of Iron, chelates out $Fe^{3+}$ from the ternary complex and results in clearing zone around the bacterial colony in the blue background. Clearing zone was more prominent in CAS agar medium depleted with iron, which indicates that stimulation of siderophore production occurs when iron becomes limiting for growth. Enterobacters are among the efficient siderophore producers. Some of the 10 isolates were found to release Siderophores in CAS agar. Siderophore production and iron transport may also contribute to the plant growth activities of associated plants. Time course experiment and initial characterization lead us to characterize the optimum time of production of siderophore .From the time course experiment it is clear that siderophore production starts at 6 hours of inoculation and optimum at 72 hours and thereafter there is no increase and at 96 hours slight decrease in the amount of production of siderophore was observed.

Time course estimation of siderophore production revealed that it starts after 6 h of growth and reaches maximum level in 2-3 days. The formation of siderophore was also strongly affected by elevated temperature. These results are in accordance with (V, Singh *et al.* 2010) who reported out of 150 bacterial isolates 16 (UKA-56, 58, 60, 72, 75, 76, 78 to 80, 83, 85, 87, 88, 90, 96 and UKA-97) were found positive for siderophore production on CAS medium. Similarly, (Catherine et al., 2012) showed that out of 43 endophytic bacteria, 12 were able to produce orange halo zone on CAS medium and belongs to genera *Pseudomonas* sp and *Flavimonas oryzihabitans* .Our results are in line with

**Table 6:** Summary of the overall result

| Sl. No. | Name | Rice variety/ Endophyte type | Growth on JNFb- | Siderophore Production | NifH gene amplification | Bacterial name |
|---|---|---|---|---|---|---|
| A | BGS2 | Bala Gora /SE | Yes | Yes | Yes | *Pantoea sp. BSJ2* |
| B | BGS3 | Bala Gora /SE | Yes | Yes | Yes | *Pantoeastewartii* |
| F | KHS1 | Kharanai /SE | Yes | Yes | Yes | Pseudomonas sp. |
| G | KHR1 | Kharanai / RE | Yes | Yes | Yes | *Sphingomonas* sp. LK11 |
| H | KHR2 | Kharanai / RE | Yes | Yes | Yes | *Pantoeaananatis* |
| I | SGS1 | Sada Gora /SE | Yes | Yes | Yes | *Burkholderiacepacia* |
| J | SGS2 | Sada Gora /SE | Yes | Yes | Yes | *Xanthomonassacchari* |
| K | SGS3 | Sada Gora /SE | Yes | Yes | Yes | *Pseudomonas* sp. |
| L | KGS2 | Kala Gora /SE | Yes | Yes | Yes | *Pantoea* sp. |
| M | KGR2 | Kala Gora / RE | Yes | Yes | Yes | *Agrobacterium tumefaciens* |

finding of (Waschutza *et al.* 1992), who demonstrated two endophytes *viz. Bacillus cereus* MQ23 and MQ23II isolated from *Sophora alopecuroides* root nodules were able to produce siderophores on CAS medium. Similarly, also documented that out of 63 bacterial isolates from the rhizosphere and rhizoplane of rice and maize plants, 20 were found positive for siderophore production. The production of siderophore offers a mechanism of plant growth promotion by chelation of Fe. Clear zone was more prominent in CAS agar medium depleted with iron, which indicates that stimulation of siderophore production occurs when iron becomes limiting for growth (Loaces *et al.* 2011). Enterobacters are among the efficient siderophore producers. The 10 isolates were found to release siderophores in CAS- Agar. Siderophore production and iron transport may also contribute to the plant growth activities of associated plants, clearing zone becomes more prominent in CAS-agar media depleted with Fe, which indicates that stimulation of siderophore production occurs when iron becomes the limiting factor for growth. Catecholate type of siderophores have been reported for *Azospirillum* and *Rhizobium*. Moreover, synthesis of phenolatesiderophores has been demonstrated in *Klebsiella oxytoca* and *Azotobacter vinelandii*. *Azospirullum brasiliense* has been reported for biosynthesis of its siderophore, spirolobactin (Sayyed *et al.* 2005). Presence of siderophore synthesis ability in these diazotrophic bacteria seems more important with reference to study, which reports that a catecholate type siderophore plays a key role in transport of Mo under iron starved condition in *Azospirillum lipoferum* (Singh *et al.* 2010) Because nitrogen-fixing bacteria require iron and Mo to convert molecular nitrogen to ammonia, the role of siderophore is very important.

All the 10 bacterial isolates were subjected to primary characterization. On the basis of morphological appearance, they were found to be diverse. Almost all bacterial isolates in this investigation were gram-negative rods 9 only one was Gram + ve (Table 5) commonly occurring chemoorgano heterotrophic eubacteria, many of which have agricultural value. Our results are agree with the findings of ( Mbai *et al.* 2013) who reported among 66 endophytic bacteria from rice root were Gram

negative as well as catalase positive. Whereas 41 (62.12%), 33 (50%) and 8 (12.12%) showed positive for citrate utilization, MR and VP test, respectively. Similarly, (Kannan *et al.* 2014) reported that out of 16 endophytic bacteria from mango root, Gram positive bacteria (68.75%) were more than Gram negative (31.25%). The colony colour was found yellowish to creamy yellow and white to creamy white. These results are in close agreement with (Krishnan *et al.* 2014) who stated that among 31 endophytic bacteria from different plant sources such as rice, *Trianthema, Agave, Opuntia, Aloe*, green gram, *Tribulus*, cotton and coconut also were Gram positive and tentatively assigned as *Bacillus* sp on the basis of morphological and biochemical characteristics.

In this study, 16S r DNA was amplified in selected 10 isolates with a pair of primer designed in the conserved region of the gene and digested with a tetra cutting restriction endonucleases in order to reveal the restriction fragment length polymorphism of 16S r DNA. On the basis of maximum % similarity among the isolates BGS2, BGS3, BGS6, BGR7, BGR5, BGR10, BGR12, SGR1, SGR3, SGR2, SGS1, SGS2, SGS3, KHS1, KGS2 were identified as *Pantoea sp.* BSJ2*, Pantoea stewartii, Rhizobium sp., Gammaproteobacteria bacterium, Mycobacterium sp. Pseudomonas sp., Sphingomonas sp.* LK11, *Pantoea ananatis, Burkholderia cepacia, Xanthomonas sacchari, Pseudomonas sp., Pantoea sp., and Agrobacterium tumefaciens.*

In this study, fidelity of the amplified segment of *nifH* was evident from the sequence, which displayed similarity with *nifH* sequence of present in database of Gene Bank. Our result are in accordance with who observed that out of 35 bacterial isolates, only 11 isolates showed *nif*H gene amplification with expected band size 300–400 bp. and with other result which administers the presence of *nif*H in total 73 potential isolates yielded the expected band size of 360bp on agarose gel.

## CONCLUSION

From the above results and discussion it is clear that the isolated endophytic bacteria all are diazotrophic and out of them 10 isolates gave +ve test for siderophore production. The isolates showed the presence of catecholate type and hydroxamate type of siderophore which will lead to another way of classification of the bacteria, *i.e,* through siderotyping (Zhao *et al.* 2011). The distribution of the siderophore producing endophytic diazotrophic bacteria is more in stem in comparison to root revealing the fact that there is translocation of the bacteria to the growing region .Therefore, this study indicates that the indigenous rice variety with ethnomedicinal property harbors endophytic diazotrophic bacteria with siderophore producing ability which can allow the plants to grow in Fe deficient medium which is the first report in this area. 10 isolates BGS2, BGS3, KHS1, KHR1, SGS1, SGS2, SGS3, KGR2, KGS1 gave +ve test for siderophore production. Theses endophytes were characterised at molecular level (16 S rDNA) and the strains identified were *Pantoea sp.* BSJ2*, Pantoea stewartii, Pseudomonas sp., Sphingomonas sp.* LK11, *Pantoea ananatis, Burkholderia cepacia, Xanthomonas sacchari, Pseudomonas sp., Pantoea sp., and Agrobacterium tumefaciens.*

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