PUBLICATIONS


Isolation and Characterization of Siderophore from Cowpea Rhizobium (Peanut Isolate)

R.S. Jadhav and A.J. Desai
Department of Microbiology and Biotechnology Centre, M.S. University of Baroda, Baroda, Gujarat, India

Abstract. In an iron-depleted broth culture of cowpea Rhizobium (a peanut isolate), phenolate type of compounds were detected. Chemical characterization showed the presence of 2,3-dihydroxy benzoic acid (DHBA) and 3,4-DHBA in the siderophore extract. Lysine and alanine were identified as conjugated amino acids of the siderophore. Maximum concentration of the siderophore in the culture supernatant was found after 24 h of growth. The compounds in the extracted siderophore induced growth of Rhizobium in a medium containing EDTA. Addition of lysine and alanine in the growth medium (20 mM each) led to a fourfold increase in siderophore production.

Iron is an essential but elusive metal for earth's life system. The hydroxides of ferric iron have a very low solubility product constant \(10^{-38} M\), making them almost insoluble. The ferrous state is comparatively more soluble. Since most of the iron in the earth's crust is in the ferric form, most of the aerobic organisms have developed an efficient means for solubilizing and transporting this ferric iron. The mechanism involves the role of low-molecular-weight organic compounds that have ability to chelate iron and transport it across the cell. These compounds are called siderophores [4]. Basically, siderophores are considered to be of two types, viz.,

a) secondary hydroxamic acid and
b) catecholate type [11]. Recent studies show that these siderophores are also capable of transporting molybdenum in Azospirillum [16] and cowpea Rhizobium [10]. Here we present a report on the characterization of the siderophore from cowpea Rhizobium.

Materials and Methods

Bacterial culture and growth conditions. Cowpea Rhizobium was isolated locally from the root nodules of the peanut (Arachis hypogea) plant. The culture was maintained on yeast extract mannitol agar containing congo red. For siderophore production, a synthetic medium with the following composition was used, per liter: 10 g mannitol, 2 g sodium glutamate, 0.5 g K$_2$HPO$_4$, 0.2 g MgSO$_4$ • 7H$_2$O, and 0.1 g sodium chloride. The medium was prepared in double glass-distilled water, and care was taken to use metal-free glassware.

Estimation and extraction of siderophore. Determination of catecholate type of siderophore in culture supernatant was done by Arnow's method [2]. For siderophore extraction, a synthetic medium with the following composition was used, per liter: 10 g mannitol, 2 g sodium glutamate, 0.5 g K$_2$HPO$_4$, 0.2 g MgSO$_4$ • 7H$_2$O, and 0.1 g sodium chloride. Medium was prepared in double glass-distilled water, and care was taken to use metal-free glassware.

Characterization of siderophore. Identification of dihydroxy benzoic acids was done by TLC with a solvent system containing benzene : toluene : acetic acid \(2 : 2 : 1\). By preparative TLC, bands corresponding to 2,3-DHBA and 3,4-DHBA were scraped out, and the UV spectrophotometric scanning of the compounds present in them was carried out with a Shimadzu spectrophotometer UV-260.
Identification of conjugated amino acids. The siderophore extract was taken and hydrolyzed with 6 N HCl. It was neutralized with 1 N NaOH, and the identification of amino acids in the sample was done by paper chromatography with a solvent system containing butanol:acetic acid:water (12.3:5).

The siderophore-mediated iron transport was studied by a method described by Modi et al. [16]. The siderophore bioassay was done by a method described by Modi et al. [10].

Results and Discussion

The culture supernatant, when tested with 1 mM FeCl₃, gave a wine color, indicating phenolate type of compounds in the culture supernatant. For the detection of the hydroxamate type of siderophore, the Gibson and Magrath method [6] was used, but hydroxamate type of compounds could not be detected in the culture supernatant.

The siderophore production was found to start after 4 h of growth, and maximum concentration was reached after 22–24 h of growth, i.e., when the culture just entered the stationary phase (Fig. 1). A similar type of pattern of siderophore production has been reported in Azospirillum [16], in which maximum siderophore production was found after 20 h of growth. Pyoverdine production in Pseudomonas aeruginosa PAO 1 was also found to be maximum after 40 h of growth [3]. In all these cases, the maximum siderophore concentration in the culture supernatant was seen at a time when the culture just entered late log phase and siderophore production occurred parallel with growth. When total DHBA concentration was checked in the growth medium (Arnow's method), it was found to be 30 µg/ml culture supernatant. The production of DHBAs was found to be dependent on the iron level in the growth medium. These compounds were formed only in the iron-starved conditions. When iron was supplemented in the growth medium (10 µM), there was no DHBA production (Table 1).

TLC of the extracted siderophore sample was carried out, and then the spots were developed with...
Hathway’s reagent (K-ferricyanide, 0.3 g; FeCl₃, 0.3 g; distilled water, 100 ml). Two clear blue spots developed. The position of one spot matched that of authentic 2,3-DHBA, and the position of the other spot exactly matched that of authentic 3,4-DHBA (Table 2).

Preparative TLC of the extracted siderophore was carried out, and bands corresponding to 2,3-DHBA and 3,4-DHBA were scraped out; the compounds eluted from them were analyzed by UV spectrophotometric scanning. UV spectra of these compounds exactly matched those of authentic 2,3-DHBA and 3,4-DHBA (Figs. 2, 3). This confirmed the presence of 2,3-DHBA and 3,4-DHBA in the siderophore extract. Earlier only 2,3-DHBA had been reported in the siderophore extract of cowpea *Rhizobium* [10].

When acid-hydrolyzed siderophore was analyzed by paper chromatography, it was found to contain alanine and lysine (Table 3). Amino acids are known to be conjugated with DHBAs in the number of siderophores reported earlier. In different cultures, different amino acids have been reported to be conjugated with DHBA. In one of the isolates of *cowpea Rhizobium*, glycine and threonine have been reported to be the conjugated amino acids of 2,3-DHBA in the siderophore [10].

When lysine and alanine were added to the growth medium, there was an increase in siderophore production. At a 20 mM concentration of each of these amino acids in the medium, there was an

### Table 2. Identification of dihydroxy benzoic acids in siderophore extract by TLC

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3-DHBA</td>
<td>0.48</td>
</tr>
<tr>
<td>3,4-DHBA</td>
<td>0.32</td>
</tr>
<tr>
<td>Siderophore extract</td>
<td></td>
</tr>
<tr>
<td>Spot 1</td>
<td>0.32</td>
</tr>
<tr>
<td>Spot 2</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Fig. 2. UV spectrophotometric scanning of compound 1 purified from siderophore extract by TLC. A, compound 1 from siderophore extract; B, authentic 2,3-DHBA.

Fig. 3. UV spectrophotometric scanning of compound 2 purified from siderophore extract by TLC. A, compound 2 from siderophore extract; B, authentic 3,4-DHBA.
Table 3. Identification of amino acids in the siderophore extract by paper chromatography

<table>
<thead>
<tr>
<th>Standard amino acids</th>
<th>R	extsubscript{f} value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>0.21</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.36</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.27</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.33</td>
</tr>
<tr>
<td>Hydrolysate of siderophore</td>
<td></td>
</tr>
<tr>
<td>Spot 1</td>
<td>0.21</td>
</tr>
<tr>
<td>Spot 2</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Table 4. Effect of amino acid addition to growth medium on siderophore production

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Siderophore production (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal medium</td>
<td>30.2</td>
</tr>
<tr>
<td>Normal medium + alanine and lysine (5 mM each)</td>
<td>47.04</td>
</tr>
<tr>
<td>Normal medium + alanine and lysine (10 mM each)</td>
<td>75.2</td>
</tr>
<tr>
<td>Normal medium + alanine and lysine (20 mM each)</td>
<td>113.1</td>
</tr>
</tbody>
</table>

* Medium as described in Materials and Methods.

almost fourfold increase in the siderophore production (Table 4). This suggests that these amino acids are possibly used as readily available precursors for siderophore biosynthesis. Similar results have been reported in the case of *Aerobacter aerogenes*, where addition of lysine to the growth medium increases aerobactin production twofold [1].

In order to study the role of the siderophore in iron transport, a bioassay was carried out in solid medium containing EDTA, which tends to chelate the iron from the medium, making it unavailable to the organism. As depicted in Fig. 5, the zone of growth exhibition was observed only around the well containing the siderophore extract. The rest of the plate showed no growth. The results led us to conclude that siderophore from the well tends to diffuse in the medium surrounding the well, quenching out iron from EDTA and making it available to the test organisms. The data were further supported by the experiment which showed enhancement of iron transport by the siderophore extract (Fig. 4). A similar type of enhancement of growth and iron transport by the siderophore has been reported for *Azospirillum* [16]. In our experiment we used authentic 2,3-DHBA and 3,4-DHBA for comparison. These two compounds also enhanced iron transport, but the
siderophore was found to be more efficient than these two authentic compounds. The conjugation of amino acids with DHBA in our siderophore might be responsible for the increased efficiency of the compound in iron transport, as it has been reported in the case of azotobactin and azotocin, where unconjugated DHBA does not promote significant iron uptake whereas the conjugated azotobactin and azotocin could promote iron uptake in <i>Azotobacter vinelandii</i> [8].

**Literature Cited**


Role of siderophore in iron uptake in cowpea Rhizobium GN1 (peanut isolate): Possible involvement of iron repressible outer membrane proteins

R.S. Jadhav and Anjana Desai *

Department of Microbiology and Biotechnology Centre, Faculty of Science, M.S. University, Baroda-390 002, Gujarat, India

(Received 25 September 1993; revision received and accepted 25 October 1993)
Subscription data

Journals are sent by surface delivery to all countries, except the following countries where SAL air delivery (Surface Airlifted Mail) is ensured: USA, Canada, Japan, Australia, New Zealand, P.R China, India, Israel, South Africa, Malaysia, Singapore, South Korea, Taiwan, Pakistan, Hong Kong, Brazil, Argentina, Mexico and Thailand

Subscription orders can be entered only by calendar year (January–December), and should be sent to: Elsevier Science B.V., Journals Department, PO Box 211, 1000 AE Amsterdam, The Netherlands, telephone (020) 5803 642, fax (020) 5803 598, or to your usual subscription agent (not for personal subscriptions). Claims for missing issues should be made within six months of publication, otherwise they cannot be honoured free of charge Subscriptions are accepted on a prepaid basis only, unless different terms have previously been agreed upon.

Members of Societies affiliated to FEMS may obtain certain FEMS Microbiology Journals at special rates. Please contact the Publisher for further information and conditions (fax +31-20 5803342).

In the USA and Canada: All questions arising after acceptance of a manuscript by the editor, especially those relating to proofs, publication and reprints, should be directed to the publishers, Elsevier Science B.V., PO Box 1527, 1000 BM Amsterdam, The Netherlands. For further information contact: Elsevier Science Publishing Co., Inc. Attn: Journal Information Center, 655 Avenue of the Americas, New York, NY 10010. Tel.: (212) 633 3750.

Back issues: Obtainable from 1981 onwards; prices are available on request. Orders may be sent to: Elsevier Science B.V., Special Services, PO Box 211, 1000 AE Amsterdam, The Netherlands.

Advertising information
Advertising orders and enquiries should be sent to (i) The Netherlands. Elsevier Science, Advertising Department, PO Box 211, 1000 AE Amsterdam; Tel. (20) 515 3220 Fax (20) 693 3041; (ii) UK: TG Scott and Son Ltd., 21 Narborough Road, Cosby, Leicestershire LE9 7TA, Tel. (0533) 763 333 Fax 0533 760 522, attn. Tim Blake; (iii) USA & Canada: Weston Media Associates, Daniel Upner, PO Box 1110, Greens Farms, CT 06436-1110, USA, Tel. (203) 261 2500 Fax (203) 261 0101.

ADONIS
This journal is in the ADONIS Service, whereby copies of individual articles can be printed out from CD-ROM on request. An explanatory leaflet can be obtained by writing to ADONIS B.V., PO Box 839, 1000 AV Amsterdam, The Netherlands.
Role of siderophore in iron uptake in cowpea *Rhizobium* GN1 (peanut isolate): Possible involvement of iron repressible outer membrane proteins

R.S. Jadhav and Anjana Desai *

*Department of Microbiology and Biotechnology Centre, Faculty of Science, M S University, Baroda-390 002, Gujarat, India*

(Received 25 September 1993, revision received and accepted 25 October 1993)

**Abstract**

Siderophore produced by cowpea *Rhizobium* GN1 (peanut isolate) was shown to be involved in iron uptake by this organism. Siderophore enhanced iron uptake in iron-starved cells. SDS-PAGE analysis of the outer membrane proteins showed two iron repressible outer membrane proteins with approximate molecular mass of 80 kDa and 76 kDa. A siderophore non-producing mutant, which was unable to grow on a medium containing synthetic iron chelators unless and until iron was added exogenously in the medium, could use siderophore of the wild-type for iron uptake indicating that the receptor for Fe-siderophore complex was intact in the mutant.

**Key words**

*Rhizobium* GN1; Siderophore

**Introduction**

Though iron is plentiful in soil, it is not freely available to microorganisms at physiological pH. To combat this low solubility most microorganisms have evolved high affinity iron uptake systems, involving low molecular mass organic compounds (iron chelators) termed siderophores [1]. Recently a number of siderophores from rhizobia have been characterized. *Rhizobium mellon* produces a structurally novel siderophore termed rhizobactin [2]. Studies on siderophore production by a range of root nodule bacteria showed that all the strains investigated gave a positive reaction to the chrome azurol-S assay, indicating that they excrete iron chelating compounds [3]. This report also showed that none of the strains studied produced catecholate siderophore, whereas two strains of *Rhizobium leguminosarum* produced hydroxamate type siderophore. Earlier studies from this laboratory have shown production of catecholate type of siderophore by cowpea *Rhizobium* [4]. Citrate acts as a siderophore in *Bradyrhizobium japonicum* [5]. Thus, in different rhizobia, different types of iron chelators are produced and structurally they appear to be strain-specific.

* Corresponding author.
The siderophore-iron complex is transported inside the cell by a specific, energy-dependent uptake system [6]. In Gram-negative bacteria, in addition to the production of siderophores, iron deficiency is also characterized by the appearance of one or more new major proteins in the outer membranes termed iron regulated outer membrane proteins (IROMPs) [7]. In some cases IROMPs have been shown to be Fe-siderophore receptors [8,9].

In the present study attempts have been made to show active involvement of the siderophore and possible role of IROMPs in iron uptake in cowpea *Rhizobium* GN1.

### Materials and Methods

**Bacterial strain**
Cowpea *Rhizobium* GN1 was isolated locally from root nodules of peanut (*Arachis hypogea*). The culture was maintained on yeast extract mannitol agar containing Congo red.

**Extraction and estimation of siderophore**
Siderophore extraction from culture supernatant of iron-starved culture was carried out as described earlier [4]. The siderophore was purified by preparative TLC of concentrated ethyl acetate extracts with benzene: toluene: acetic acid (2:2:1 v/v) as solvent system. The siderophore was dissolved in double glass distilled water and the concentration estimated using Arnow's method [10] with 2,3-dihydroxybenzoic acid as a standard.

**Iron uptake studies**
$^{55}$FeCl$_3$ was obtained from Bhabha Atomic Research Centre, Bombay, India. $^{55}$Fe-siderophore complex was formed by mixing $^{55}$FeCl$_3$ with approx. 4 mol excess of siderophore (stoichiometric analysis has shown that, for 1 mol of Fe, 2 mol of siderophore in terms of DHBA are required). The concentration of $^{55}$Fe (either free Fe or Fe-siderophore complex) in the uptake buffer was 1 $\mu$M. For uptake studies bacterial cells were grown in either iron-deficient or iron-sufficient Ashby's mannitol broth. Cells from mid-log phase were collected by centrifugation (at 10000 rpm for 20 min), washed twice with the uptake buffer (5 mM morpholinoethane sulphonate buffer, pH 7.0) and suspended in the same buffer containing either only $^{55}$Fe or $^{55}$Fe-siderophore complex. The uptake study was carried out in 250-ml flasks containing 50 ml uptake buffer with approximately $10^9$ cells ml$^{-1}$. At intervals of 5 min 1 ml of the samples were removed and filtered through membrane filters (0.45 $\mu$m pore size) which had been previously soaked in the uptake buffer for 30 min and the bacteria immediately washed with 5 ml of 0.5% sodium thioglycollate. Filters were dried and counted in Ria Luma (Lumac/3M bv, the Netherlands) in liquid scintillation counter (LKB Rackbeta Spectral Scintillation Counter). All values were corrected for binding of the iron complexes to the filter.

**Isolation of outer membrane proteins**
Outer membrane proteins were isolated by the method described by Page and Huyer [11]. The membrane proteins were separated by SDS-PAGE as described by Lugtenberg [12]. Outer membrane preparations obtained by this method were compared with those obtained by sucrose gradient centrifugation; purity was checked by measuring the presence of 2-ketodeoxyoctonoate (marker for outer membrane) and the absence of NADH oxidase activity (marker for cytoplasmic membrane). The results showed that the method used gave pure outer membrane preparation (data not shown).

**Isolation of siderophore nonproducing mutant**
Siderophore mutants were isolated by treating cowpea *Rhizobium* GN1 with NTG as described by Arceneaux [13]. Mutants were screened on chrome azurol-S containing agar plates [14].

### Results and Discussion

In order to examine iron uptake in strain GN1, cells were grown under Fe-limited or Fe-sufficient conditions. When checked for siderophore-mediated iron uptake, enhanced iron uptake was
observed only in Fe-limited cells (Fig. 1). In contrast, Fe-sufficient cells showed no significant iron uptake even in the presence of siderophore (Fe-siderophore complex). In _Anabaena_ sp. the rates of uptake of ferric-Schizokinen were significantly faster in iron-starved cells than in the iron-supplemented cultures [15]. Similarly, Fe-limited cells of _Azotobacter vinelandii_ are more efficient in iron uptake than the Fe-sufficient cells [16]. In _Bradyrhizobium japonicum_, which utilises citrate as siderophore, the cells grown under iron-starved or iron-supplemented conditions showed similar patterns of iron uptake for 2 min, but the Fe-limited cells showed continued uptake over the next 25 min whereas Fe-supplemented cells did not [5].

One of the components of a siderophore mediated iron uptake system is siderophore receptor protein, synthesis of which is likely to be regulated by the iron level of the growth medium. In _cowpea Rhizobium_ GN1 we found synthesis of two outer membrane proteins with approximate molecular masses 80 kDa and 76 kDa under iron-starved conditions (Fig. 2). Since these proteins are not synthesized under Fe-sufficient conditions, it is possible that one of these proteins constitute siderophore receptor protein. This may explain siderophore-mediated enhanced iron uptake only in iron-starved cells and not in iron-sufficient cells. In _Escherichia coli_ the outer membrane receptor protein (Fep A) for enterobactin has been reported to be of molecular mass of 81 kDa [9]. Similarly, in _Pseudomonas aeruginosa_, an 80 kDa iron-regulated outer membrane polypep-
A mutant (GN1M) of cowpea *Rhizobium* GN1 defective in iron transport, was unable to produce the siderophore, but was able to utilise the wild-type siderophore. The mutant failed to produce an orange halo around the colony on CAS agar plates (data not shown). Unlike the wild-type, the mutant was unable to grow on Ashby's mannitol medium plus 200 μM bipyridyl (data not shown). The wild-type presumably grows on this medium because of its ability to produce the siderophore, which can remove iron from bipyridyl. A similar use of bipyridyl for the study of siderophore-negative mutants has been reported in *Pseudomonas putida* WCS358 and *Azotobacter vinelandii* [17,18].

Since a siderophore bioassay study showed that the mutant (GN1M) could utilise the siderophore of the parent strain, an iron uptake study with the mutant grown under iron-starved conditions was carried out. The mutant could grow under iron-limited conditions (0.3 μM) for only one transfer. In subsequent transfers the mutant failed to grow unless it was supplemented with either iron or wild-type siderophore (data not shown). An iron-uptake study (Fig. 3) showed that siderophore could enhance iron uptake in the mutant strain also.

Outer membrane protein profile of the mutant also showed two iron-regulated outer membrane proteins with approximate molecular masses of 80 kDa and 76 kDa identical to the wild-type (data not shown). Hence, as speculated earlier, one of these proteins might be acting as Fe-siderophore receptor which is responsible for enhanced siderophore-mediated iron uptake in the mutant and parent strain.

Acknowledgement

R.S.J. wishes to thank the University Grants Commission, New Delhi, India, for providing a Senior Research Fellowship during the course of this investigation.

References

Short Communication

Involvement of the siderophore of cowpea Rhizobium in the iron nutrition of the peanut

R.S. Jadhav, N.V. Thaker and A. Desai

An iron-inefficient variety of peanut plant, when grown hydroponically with the catechol siderophore of Rhizobium (peanut isolate), showed increased growth and chlorophyll content compared with plants grown with Fe alone. The siderophore, when used in concentrations less than the concentrations of Fe, was still effective in growth promotion, indicating that it might function as a shuttle agent, solubilizing and supplying Fe to the plant. Similar results were also obtained with desferrioxamine B.

Key words. Iron nutrition, peanut, siderophore

Although iron is abundant in soil (1 to 6%) it is often unavailable to plants and microorganisms because of the very low solubility of ferric hydroxides (Messenger & Ratledge, 1985). It has been suggested that various microbial siderophores, which increase and regulate iron availability in the rhizosphere, are involved in the iron nutrition of plants (Jurkevitch et al. 1988) but whether the host legume itself may benefit from rhizobial siderophore production has not been explored (Guerinot 1991). The association of rhizobia with the roots of leguminous plants leading to a beneficial effect on plant growth, is well known. Apart from fixing N₂ in the nodules, rhizobia in the rhizosphere may also promote plant growth by producing growth hormones or enhancing nutrient uptake. Siderophore production and iron transport may also contribute to the plant-growth-promoting activities of Rhizobium species. In view of this, the present investigation was designed to study the effect of a siderophore (catecholate compound) on the transport of iron in Arachis hypogea (peanut) and to compare it with the effect of desferrioxamine B, a hydroxamate siderophore.

Materials and Methods

Plant Culture

Seeds of an iron-inefficient variety of Arachis hypogea (from the Department of Crop Improvement, Gujarat Agriculture University, Gujarat, India) were surface sterilized and then germinated on moistened filter paper in sterile Petri plates. The seedlings were transferred to continuously aerated nutrient solution (in dark-brown glass pots) containing (g/l) Ca(NO₃)₂ • 4H₂O, 1.18; KNO₃, 0.31; K₂HPO₄, 0.14; MgSO₄ • 7H₂O, 0.49; H₃BO₃, 0.0029; MnCl₂, 1.018; ZnSO₄, 0.00022; CuSO₄ • 5H₂O, 0.0039% and FeCl₃, 0.0002. FeCl₃ solution was added according to the experiment. The pH of the medium was adjusted to 7.5 with 0.1M KOH and 1.0 g CaCO₃/l was added as buffering agent. Two iron chelators, the siderophore of cowpea Rhizobium and desferrioxamine B, were studied using 10 μM Fe with 10, 20, 30 or 50 μM of one of the chelators. The plants were grown in a growth chamber with a 16/8 h day/night cycle and six to eight replicates for each treatment. The growth of the plants was monitored and expressed as shoot length. Chlorophyll extraction and estimation were as described by Moore (1981). The whole experiment was repeated once.

Siderophores

The siderophore of the cowpea Rhizobium GN1 (peanut isolate) was extracted and its concentration estimated as described earlier (Jadhav & Desai 1992). Desferrioxamine B, Desferal, was from Sigma.

Results and Discussion

The variety of peanut (Arachis hypogea) tested was iron inefficient in that it showed severe symptoms of chlorosis when grown under iron-deficient conditions (data not shown). It could therefore be used to check if plants can use microbial siderophores for iron nutrition. The effect of cowpea Rhizobium siderophore, a catecholate compound (Jadhav & Desai 1992), on iron nutrition of the peanut (the host plant...
Table 1 Effect of siderophores on growth (shoot length) of peanut.

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Shoot length (cm) after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 days</td>
</tr>
<tr>
<td>Control (no external Fe)</td>
<td>04 94 ± 0 20</td>
</tr>
<tr>
<td>10 μM Fe</td>
<td>07 12 ± 0 61</td>
</tr>
<tr>
<td>10 μM Fe + 10 μM CS</td>
<td>10 00 ± 0 37</td>
</tr>
<tr>
<td>10 μM Fe + 30 μM CS</td>
<td>08 28 ± 0 41</td>
</tr>
<tr>
<td>10 μM Fe + 10 μM DFOB</td>
<td>09 70 ± 0 26</td>
</tr>
<tr>
<td>10 μM Fe + 30 μM DFOB</td>
<td>08 33 ± 0 23</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations.

CS—Calecholate siderophore of cowpea Rhizobium; DFOB—desferrioxamine B

the Rhizobium used in this study) was examined and compared with the effect of a hydroxamate siderophore (desferrioxamine B). Hydroxamates may be present in the soil so they are produced by many soil fungi (Messenger & Ratledge 1985).

With 10 μM Fe, the addition of 10 μM of either chelator gave significant increases and chlorophyll content of the peanut compared with the control (Tables 1 and 2). With excess iron chelator (siderophore of cowpea Rhizobium or desferrioxamine B) the chlorophyll content showed a gradual decrease (Table 2). Both chelators can therefore act as shuttle agents, solubilizing and mobilizing Fe for iron nutrition of plants. Stoichiometric analysis of the siderophore of the cowpea Rhizobium has shown that for 1 mol Fe, two mol siderophore are required (data not shown). Hence, when the siderophyll is used at concentrations more than double that of Fe, there will be an excess of free siderophore. The reduced chlorophyll content at higher concentrations of the siderophore of cowpea Rhizobium (Table 2) might be because the peanut absorbs Fe from the pool of solubilized, non-chelated Fe generated by conversion of Fe-siderophore to free Fe and free siderophore near the absorption site. At higher siderophore concentrations the Fe-siderophore/siderophore ratio decreases and the siderophore may then compete with the plant for the free Fe generated, thus affecting iron accessibility to the plant. Desferrioxamine B may also act in the same fashion. Cline et al. (1984) reported that desferrioxamine increased yields and decreased chlorosis in sorghum when used at lower concentrations than the concentration of Fe used.

In the soil, insoluble Fe will always be in excess compared with the concentration of siderophore. Under such conditions Rhizobium may help the host plant to survive under deprived levels of accessible/soluble Fe by producing siderophore and thereby mobilizing the insoluble Fe and making it accessible to the plant.

References


(Received in revised form 22 December 1993; accepted 23 December 1993)