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Effect of metals on a siderophore producing bacterial isolate and its implications on microbial assisted bioremediation of metal contaminated soils

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HIGHLIGHTS

- A unique catecholate siderophore producing *Bacillus amyloliquefaciens* strain.
- First report on *B. amyloliquefaciens* NAR38.1 siderophore binding to Fe^{+2} and Fe^{+3}.
- Variations induced by essential and abiotic metals on siderophore production.
- Implications of siderophore producing bacteria in improving phytoextraction.

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ABSTRACT

A bacterial isolate producing siderophore under iron limiting conditions, was isolated from mangroves of Goa. Based on morphological, biochemical, chemotaxonomical and 16S rDNA studies, the isolate was identified as *Bacillus amyloliquefaciens* NAR38.1. Preliminary characterization of the siderophore indicated it to be catecholate type with dihydroxy benzoate as the core component. Optimum siderophore production was observed at pH 7 in mineral salts medium (MSM) without any added iron with glucose as the carbon source. Addition of NaCl in the growth medium showed considerable decrease in siderophore production above 2% NaCl. Fe^{+2} and Fe^{+3} below 2 μM and 40 μM concentrations respectively, induced siderophore production, above which the production was repressed. Binding studies of the siderophore with Fe^{+2} and Fe^{+3} indicated its high affinity towards Fe^{+3}. The siderophore concentration in the extracellular medium was enhanced when MSM was amended with essential metals Zn, Co, Mo and Mn, however, decreased with Cu, while the concentration was reduced with abiotic metals As, Pb, Al and Cd. Significant increase in extracellular siderophore production was observed with Pb and Al at concentrations of 50 μM and above. The effect of metals on siderophore production was completely mitigated in presence of Fe. The results implicate effect of metals on the efficiency of siderophore production by bacteria for potential application in bioremediation of metal contaminated iron deficient soils especially in the microbial assisted phytoremediation processes.

1. Introduction

Siderophores, iron chelating agents, are produced by microorganisms under iron limiting conditions (Dhaenens et al., 1999; Vraspir and Butler, 2009; Braud et al., 2009a). Although iron is the key factor in regulating siderophore production, other factors such as pH, temperature, carbon source and other metals play an important role (Saha et al., 2012). While siderophores have an extremely high affinity for ferric iron they also form complexes with metals other than Fe^{3+}, although, with a lower affinity (Braud et al., 2009a). Metals other than iron are also reported to stimulate or repress siderophore production in bacteria (Braud et al., 2010). The production of siderophore by bacteria in presence of toxic metals implicates their potential in uptake, mobilization of heavy metals or developing metal resistance. Binding of siderophores to metals reduces the free metal concentration thereby restricting their diffusion across the porins. However, in the case where the receptor fails to recognize the actual complex of iron and siderophores, it can lead to intracellular metal accumulation (Schalk et al., 2011).

Heavy metal pollution of soils is a serious problem and demands efficient clean up of the polluted areas (McGrath et al., 1995). Most of the conventional methods used for soil remediation

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are uneconomical and result in deterioration of soil texture and its organic content (Rajkumar et al., 2010). One of the emerging technologies for bioremediation of metal contaminated soils is "microbial assisted phytoremediation", a process of utilizing plants to absorb, accumulate and detoxify contaminants in soil through physical, chemical and biological processes (Prasad et al., 2010) in the presence of Plant Growth Promoting Rhizobacteria (PGPR). These rhizhosphere bacteria are known to support the growth and sustenance of plants. Some of these bacteria are also metal resistant paving a way for the benign technology for reclamation of metal polluted soils. An important characteristic of PGPR for use as a bio-inoculant in this technology is their ability to produce siderophores (Jing et al., 2007). Therefore, for such a strategy to be viable, a better understanding of siderophore producing bacteria and their interaction with metals is required.

During our studies on siderophore producing bacteria from coastal ecosystems (Gaonkar et al., 2012), we isolated a *Bacillus* culture from mangrove sediments showing production of a siderophore in iron deficient conditions. We report here the characterization of the bacterial isolate and the alterations implicated by metals on growth and siderophore production. The metals selected were those which are essential for the metabolic activity like Zinc (Zn), Cobalt (Co), Copper (Cu), Molybdenum (Mo) and Manganese (Mn) and those which were non-essential toxic metals, termed as abiotic (Schalk et al., 2011), mainly, Arsenic (As), Lead (Pb), Aluminium (Al) and Cadmium (Cd).

2. Materials and methods

2.1. Isolation and identification of the isolate

Bacterial strain used in this study was isolated from sediment sample obtained from mangroves located at Ribander, Goa. The production of siderophore by the isolate was determined using Chrome azurol sulphonate (CAS) assay as described by Schwyn and Neiland (1987). To prepare 100 ml of CAS solution, 60.5 mg of CAS was dissolved in 50 ml of deionised water to which 10 ml of $FeCl_3{\cdot}6H_2O$ solution was added. 72.9 mg HDTMA (Hexadecyl Trimethyl Ammonium bromide) dissolved in 40 ml of deionised water was added to CAS to make the volume to 100 ml. The selected isolate NAR38.1 was grown on nutrient agar and its cultural, morphological and biochemical characteristics were noted. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 8F and 1492R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 1413 bp 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The 16 S rDNA sequence was used to carry out BLAST (Altschul et al., 1990) with the nrdatabase of NCBI genbank database. Sequences were selected and aligned using multiple alignment software program Clustal X (Thompson et al., 1997). Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4.2.1. Chemotaxonomic characterization by FAME analysis was performed by Gas Chromatography using MIDI Sherlock Microbial Identification System software, version 6.1.

2.2. Characterization of siderophore

All the glassware used in the study were soaked in 6 M HCl overnight and then repeatedly washed with deionised water to remove any metal traces. Mineral salts medium (MSM) (Parulekar and Mavinkurve, 2006) with 0.2% glucose was used for growth and siderophore production. The isolate was inoculated in MSM without $FeSO_4$ and incubated on shaker at 150 rpm and 28 °C for 24 h, the culture broth centrifuged at 4480 g for 10 min and the supernatant was subjected to following tests for determination of the functional group: Hydroxamate group was checked using Neiland's spectrophotometric assay (Neilands, 1981), Tetrazolium salt test (Snow, 1954) and Csaky assay (Gilliam et al., 1981). For Catecholate nature Neiland's spectrophotometric assay (Neilands, 1981) and Arnow's assay (Arnow, 1937) were used. Carboxylate nature was tested using Vogel's chemical test (Yeole et al., 2001) and spectrophotometric assay (Shenker et al., 1992).

2.3. Effect of pH and NaCl on growth and siderophore production

Effect of NaCl (1–5%) and pH (5–9) on growth and siderophore production was determined using MSM with variations in pH/NaCl and analyzed after 24 h of incubation. Growth was measured as increase in turbidity at 600 nm using a Shimadzu UV–Visible spectrophotometer (UV-2450). For siderophore analysis the supernatant was centrifuged at 4480 g for 10 min and cell free supernatant was analyzed using Arnow's test (Arnow, 1937). The presence of catechol was noted by the development of a red color and an increase in absorbance at 515 nm. The concentration of siderophore was determined using catechol as the standard.

2.4. Effect of iron on siderophore production

Flasks containing MSM with 0.2% glucose as the sole carbon source, supplemented with iron, in increasing concentrations (Fe^{+3} = 5, 10, 20, 30, 40 and 50 μM; Fe^{+2} = 1, 2, 3, 4 and 5 μM) were inoculated with 5% of exponential cells grown in the respective medium. All the culture flasks were incubated at 150 rpm at 28 °C and growth and siderophore production was monitored over a period of 72 h as described above.

To determine binding of siderophore to Fe^{+2} and Fe^{+3}, the siderophore was extracted from cell free supernatant of overnight grown culture. The pH of the supernatant was adjusted to pH 2 and siderophore was extracted with one-fifth the volume of ethyl acetate. The ethyl acetate was evaporated to obtain crude siderophore (Nair et al., 2007). The siderophore was dissolved in deionised water at a concentration of 10 μg/ml. To 0.5 ml of siderophore, 0.5 ml of Fe^{+2} (0.0001, 0.0005, 0.001, 0.005, 0.01 and 0.05 M) or Fe^{+3} (0.0001, 0.0005, 0.001, 0.005, 0.01 and 0.05 M) was added and the solution was centrifuged at 6797 g for 10 min to remove iron bound siderophore. The unbound siderophore in the solution was estimated using Arnow's test.

2.5. Effect of metal concentration on growth and siderophore production

Stock solutions of following metal forms: $MnSO_4$, $Co(NO_3)_2{\cdot}6H_2O$, $ZnSO_4{\cdot}7H_2O$, $Na_2HAsO_4{\cdot}7H_2O$, $Pb(NO_3)_2$, $Al_2(SO_4)_3{\cdot}16H_2O$, $CuSO_4$, $CdSO_4{\cdot}8H_2O$ and $Na_2MoO_4{\cdot}2H_2O$ were prepared and sterilized with 0.22 μM filters under aseptic conditions. These stock solutions were incorporated in the sterile medium (MSM without $FeSO_4{\cdot}7H_2O$) to adjust metal concentration to predetermined levels. Mn, Mo and Zn were tested at 125, 250, 500 and 1000 μM and Co, Cu, Cd, As, Pb and Al were tested at lower concentrations of 10, 20, 50 and 100 μM. The flasks were inoculated with 5% of exponential cells grown in MSM without any metal ions. All the culture flasks were incubated at 150 rpm at 28 °C and growth and siderophore production was monitored at 24 h as mentioned above.

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3. Results and discussion

3.1. Isolation and identification of the isolate

The mangrove ecosystem is a nutrient rich, ecologically balanced ecosystem. The recycling of nutrients is mainly brought about by different groups of microorganisms following the detrital type of food chain. Due to a large number of bacteria playing a role in recycling and mineralization, this ecosystem is prone to be deficient in essential elements such as iron (Bashan and Holguin, 2002). An attempt to isolate siderophore producers from a mangrove ecosystem by plating the samples directly on CAS agar plates resulted in the isolation of very few cultivable microorganisms, probably due to the toxicity of HDTMA (Yoon et al., 2010). The samples were therefore plated on nutrient agar and the isolates were then screened for siderophore production.

One of the isolates showed a distinct zone on the CAS medium indicating the production of the siderophore in copious amounts and was selected for further studies. The isolate was found to be Gram-positive, rod shaped, catalase and oxidase positive, utilized citrate and fermented glucose. The dominant fatty acids determined by FAME analysis were 14:0 iso (0.99%), 15:0 iso (20.43%), 15:0 anteiso (41.94%), 16:0 (9.71%), 16:1 w11c (1.79%), 17:1 iso w10c (1.12%), 17:0 iso (6.11%) and 17:0 anteiso (8.47%). Based on the above results, the culture was tentatively identified as *Bacillus* sp. using Bergey's Manual of Systematic Bacteriology (Sneath et al., 1986). The identification was confirmed with 16S rDNA sequencing showing closest match to *Bacillus amyloliquefaciens strain Y26* (GenBank Accession Number: JQ798394.1) (Fig. 1). The sequence deposited in the gene bank has an accession number as JX555984.

B. amyloliquefaciens appeared on the Approved Lists of Bacterial Names only in the year 1987 although it was identified in 1943. Because of its close affinity with *Bacillus subtilis*, the organism was given subspecies status as "*B. subtilis* subsp. *amyloliquefuciens*" or was included in *B. subtilis* as a variant that produces copious quantities of extracellular enzymes (Priest et al., 1987). However, a number of molecular and other evidences have proved that *B. amyloliquefaciens* is distinct from *B. subtilis*. Deoxyribonucleic acids (DNAs) from strains of *B. amyloliquefaciens* share less than 25%, 13%, and 5% homology with DNAs from strains of *B. subtilis*, *B. licheniformis*, and *B. pumilus*, respectively (Priest et al., 1987). *B. amyloliquefaciens* is now known to colonize plant root surfaces and is distinguishable from *B. subtilis* by its abilities to stimulate plant growth.

3.2. Characterization of siderophore

Isolate NAR38.1 produced CAS detectable siderophore. Supernatant of the culture grown in iron free MSM when subjected to various tests to determine functional group of the siderophore showed presence of the catechol type of functional group. Such catecholate siderophores are known to be produced, among others, by

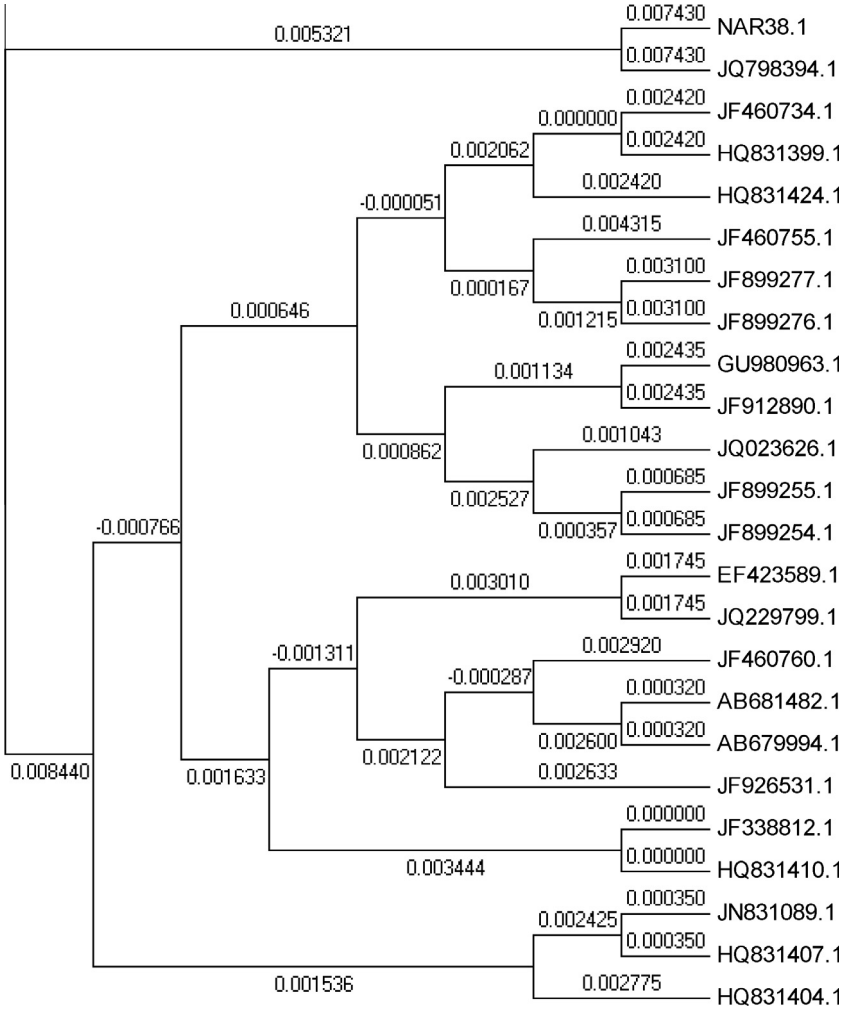

Fig. 1. Evolutionary relationships of *B. amyloliquefaciens* NAR38.1 with closely related *Bacillus* species.

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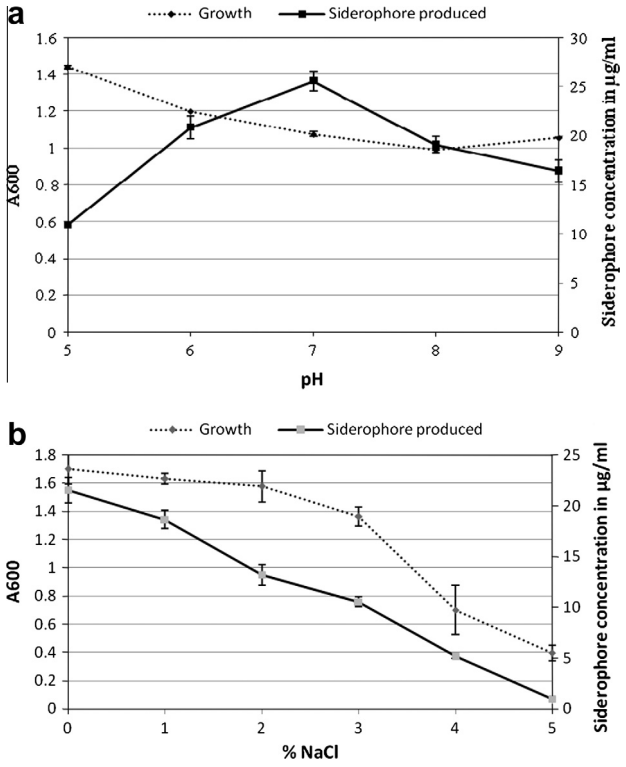

Fig. 2. (a) Effect of pH and salinity on growth and siderophore production by *B. amyloliquefaciens* NAR38.1. (b) Effect of NaCl on growth and siderophore production by *B. amyloliquefaciens* NAR38.1.

Magnetospirillum magneticum (Calugay et al., 2006), *B. subtilis* (Corynebactin), *Corynebacterium glutamicum* (Corynebactin), *Azotobacter vinelandii* (protochelin, azotochelin), *Aeromonas hydrophila* (Amonabactins), *Chryseomonas luteola* (Cheyseomonin) and *Rhodococcus erythropolis* (heterobactins) (Winkelmann, 2004).

3.3. Effect of pH and NaCl

The culture was isolated from a mangrove ecosystem where the salinity is known to range from 12% to 15% and there are micro-niches which have an alkaline/acidic pH due to the breakdown of plant litter (Agate et al., 1988). In order to understand the effect of such changes in pH and salinity the isolate was exposed to varying pH and salt levels. It was interesting to note that although the change in pH did not show any significant effect on growth of NAR38.1, the siderophore production was found to decrease under both acidic and alkaline conditions (Fig. 2a). Optimum siderophore production, as determined by Arnow's test, was observed at pH 7. Sayyed et al. (2005) have also reported pH 7 to be optimum for siderophore production, in a Gram-negative isolate, *Pseudomonas fluorescens*. The decrease in siderophore production under acidic pH could be attributed to the solubilisation of iron at lower pH resulting in iron availability (Lankford and Byers, 1973). Further, ferric complexes of catecholates are also known to be unstable at acidic pH. In such ecosystems therefore, biosynthesis of hydroxamates siderophore is preferred over synthesis of catecholates (Winkelmann, 2004). It has also been reported that at low pH destruction of siderophores is noted specially in *Pseudomonas aeruginosa* indicating that alkalinity is important to avoid siderophore destruction. Changes in pH are correlated with metabolism of the carbon source which reflects on the stability of siderophore produced by *P. aeruginosa* (Villegas et al., 2002). Characterization of the siderophore produced by the isolate NAR38.1 would throw light on the effect of pH on its stability.

Significant decrease was noted in siderophore production with increase in salt concentration up to 5% (Fig. 2b) with no effect on growth up to 2% of NaCl. However, further increase showed an effect both on growth as well as siderophore production. Such an effect on reduction in siderophore production as observed with the isolate has also been noted with siderophore producing *Rhizobium* strains nodulating *Macrotyloma uniflorum*. These strains showed increased siderophore production up to 8–9% of salt concentration,

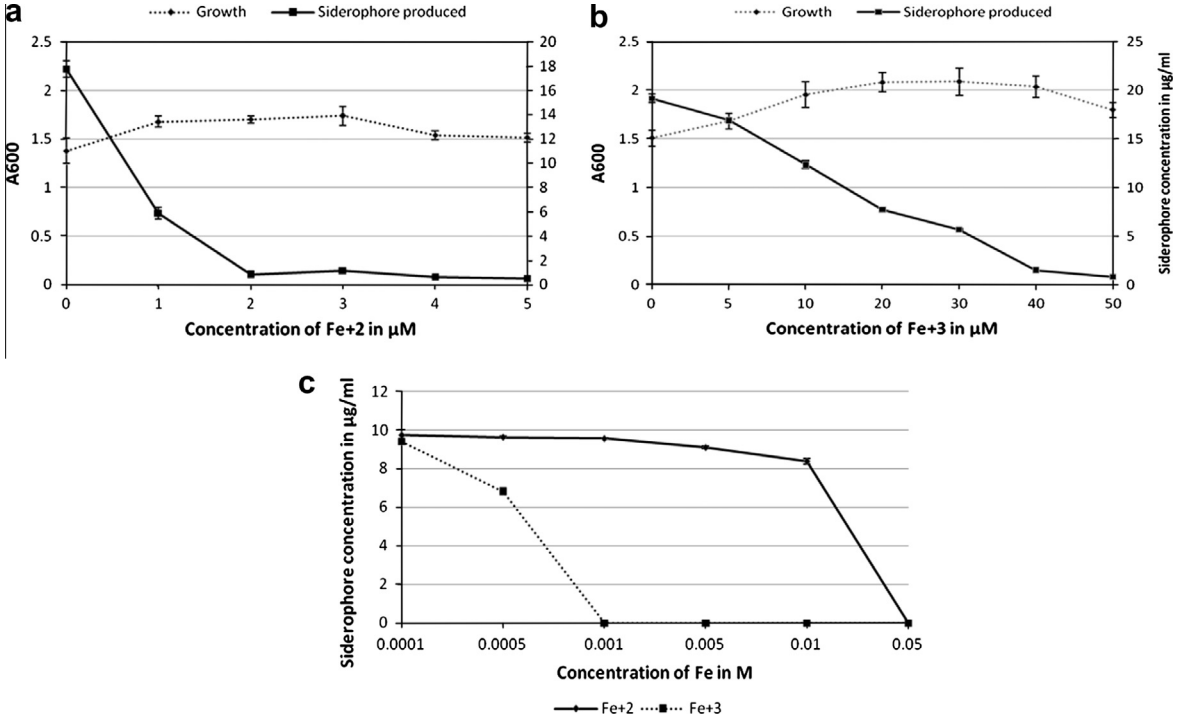

Fig. 3. (a) Effect of ferrous concentration on growth and siderophore production by *B. amyloliquefaciens* NAR38.1. (b) Effect of ferric concentration on siderophore production by *B. amyloliquefaciens* NAR38.1. (c) Binding of Fe^{+2} and Fe^{+3} to siderophore of *B. amyloliquefaciens* NAR38.1.

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but siderophore production ceased when the salt concentration was increased further (Prebhavati and Mallaiah, 2008).

3.4. Response of the isolate to iron in ferrous and ferric form

A significant change was observed in siderophore production in the presence of the divalent and trivalent iron forms. In MSM with glucose containing different levels of iron, siderophore production appeared up to 1 μM of Fe^{+2} (Fig. 3a) and up to 30 μM of Fe^{+3} (Fig. 3b), suggesting that higher levels of Fe^{+3} are required to suppress siderophore production . Fe^{+2} is a soluble form of Fe which easily diffuses across the bacterial cell membranes and is made available to the cell (Braun and Hantke, 2007). Fe^{+3}, on the other hand, is an insoluble form of Fe and is transported in the cell by siderophore. Iron is released from the siderophores by the action of dedicated enzymes that carry out the reduction of siderophore-bound Fe^{3+} to Fe^{2+} (Chu et al., 2010). This difference in the availability and mode of transport of the two forms of Fe may result in the difference in the response of the isolate. Earlier studies on the effect of iron concentration on siderophore production by *P. aeruginosa* have shown siderophore production even with 248 μM of Fe^{+3} (Villegas et al., 2002). Rachid and Ahmed (2005) have reported Fe^{+3} levels of 200 μg/ml being inhibitory to siderophore production by *P. fluorescens*. In a study by Sayyed et al. (2005) it was observed that the threshold level of iron in $FeCl_3$ form that suppresses siderophore production in *P. fluorescens* and *P. putida* is 20 μM, whereas in the present study the Gram-positive isolate showed no siderophore production above 30 μM. The requirement of iron is based on the type of bacteria as well as the nutrients and carbon sources available to the organisms (Villegas et al., 2002; Gaonkar et al., 2012).

Ferric ion is known to have a high affinity for siderophores in contrast to Fe^{+2} which binds weakly to the siderophore. This extreme difference in the affinity is important for release of Fe^{+3} from the siderophore by reducing it to Fe^{+2} (Xiao and Kisaalita, 1998). However, some of the siderophores, for an example pyoverdine,

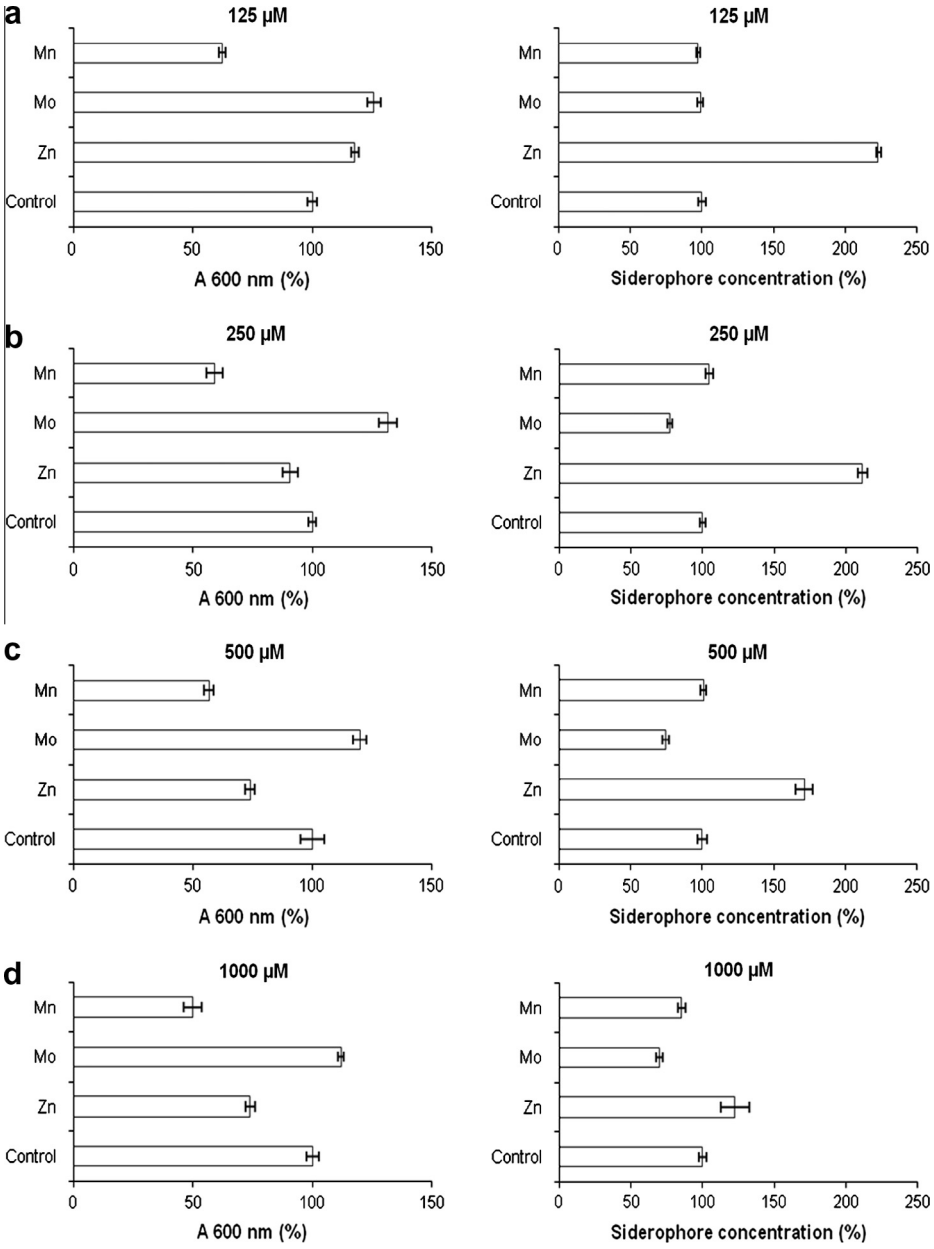

Fig. 4. Effect of Mn, Mo and Zn on growth and siderophore production by *B. amyloliquefaciens* NAR38.1.

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have been reported to bind to Fe^{+3} as well as Fe^{+2} (Xiao and Kisaalita, 1998; Gaonkar et al., 2012). In the present study, very low concentrations of Fe^{+3} (0.001 M) resulted in complete binding of the siderophore than those of Fe^{+2} (0.05 M) (Fig 3c).

3.5. Effect of metals on growth and siderophore production

Of the nine metals used for the study, divalent metals were Manganese (Mn), Cobalt (Co), Zinc (Zn), Molybdenum (Mo), Arsenic (As), Lead (Pb), Cadmium (Cd) and Copper (Cu). Aluminium (Al) was in its trivalent form. Mn, Co, Zn, Mo and Cu are essential for cellular processes and are present in bacterial cell at mM or μM quantities (Heldal et al., 1985), however, the presence of an excess of these metals could be toxic due to interactions with non-specific targets (Schalk et al., 2011). Abiotic metals used for the study were Cd, As, Pb and Al. The effect of the metals showed varied effects on the growth and siderophore production in the isolate NAR38.1.

3.5.1. Effect of essential elements in different concentrations

Zn was found to increase growth by 20% at 125 μM (Fig. 4a), with a twofold increase in siderophore concentration. While at 250 μM (Fig. 4b), there was a decrease in growth, yet a twofold increase was observed in siderophore production. Such an effect with Zn^{+2} has also been reported for *Azotobacter vinelandii* (Huyer and Page, 1988) when 20 or 40 μM of $ZnSO_4$ was added.

A significant observation was depicted with Mn wherein growth was reduced by 40% and more with increase in concentration, but no alteration in the concentration of the siderophore was seen as compared to the control (Fig. 4a–d). This indicated that at reduced growth also the same amount of siderophore (approximately 8.8 μg/ml) is produced implicating an increase in siderophore production. Mn^{+2} is known to bind to siderophores like

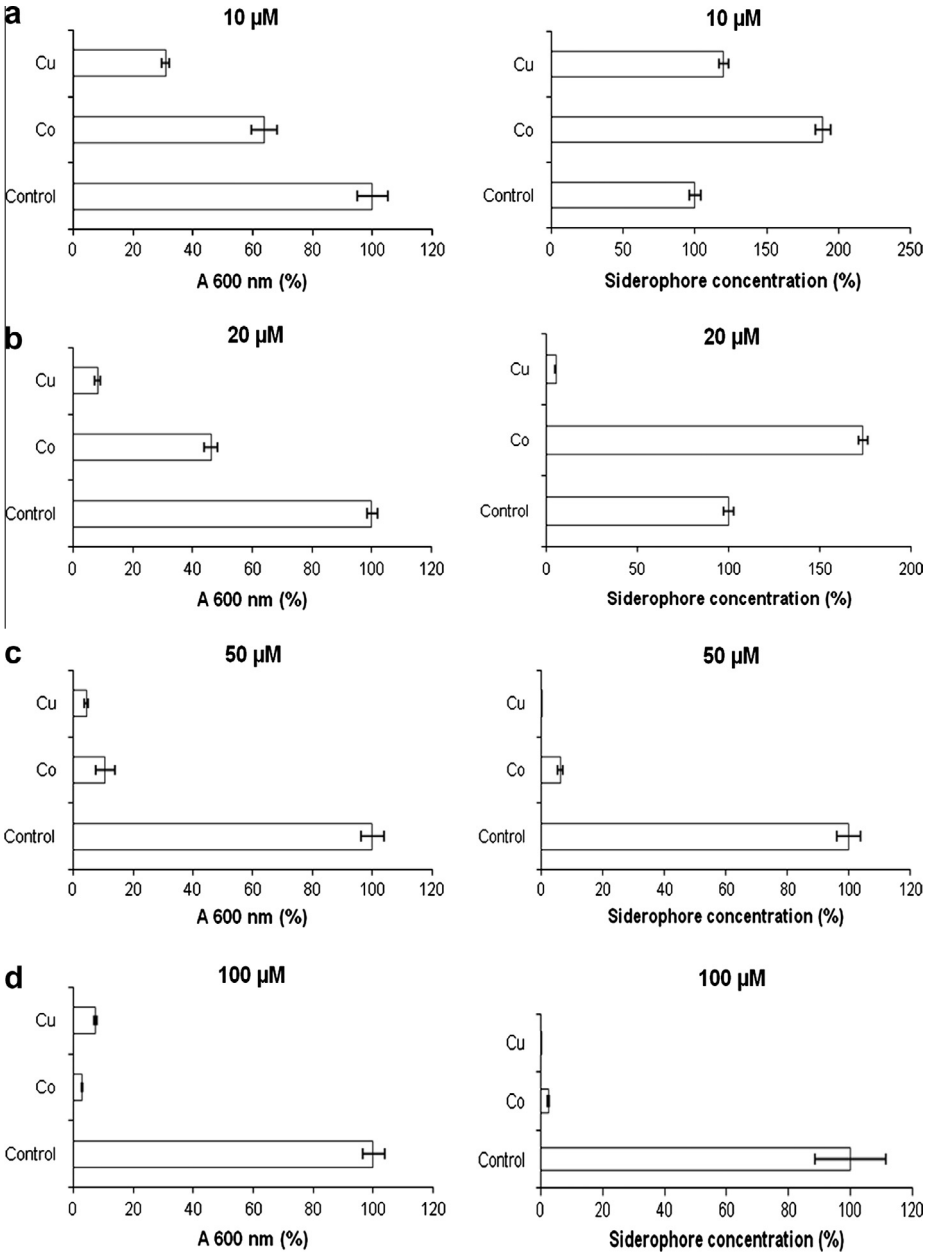

Fig. 5. Effect of Cu and Co on growth and siderophore production by *B. amyloliquefaciens* NAR38.1.

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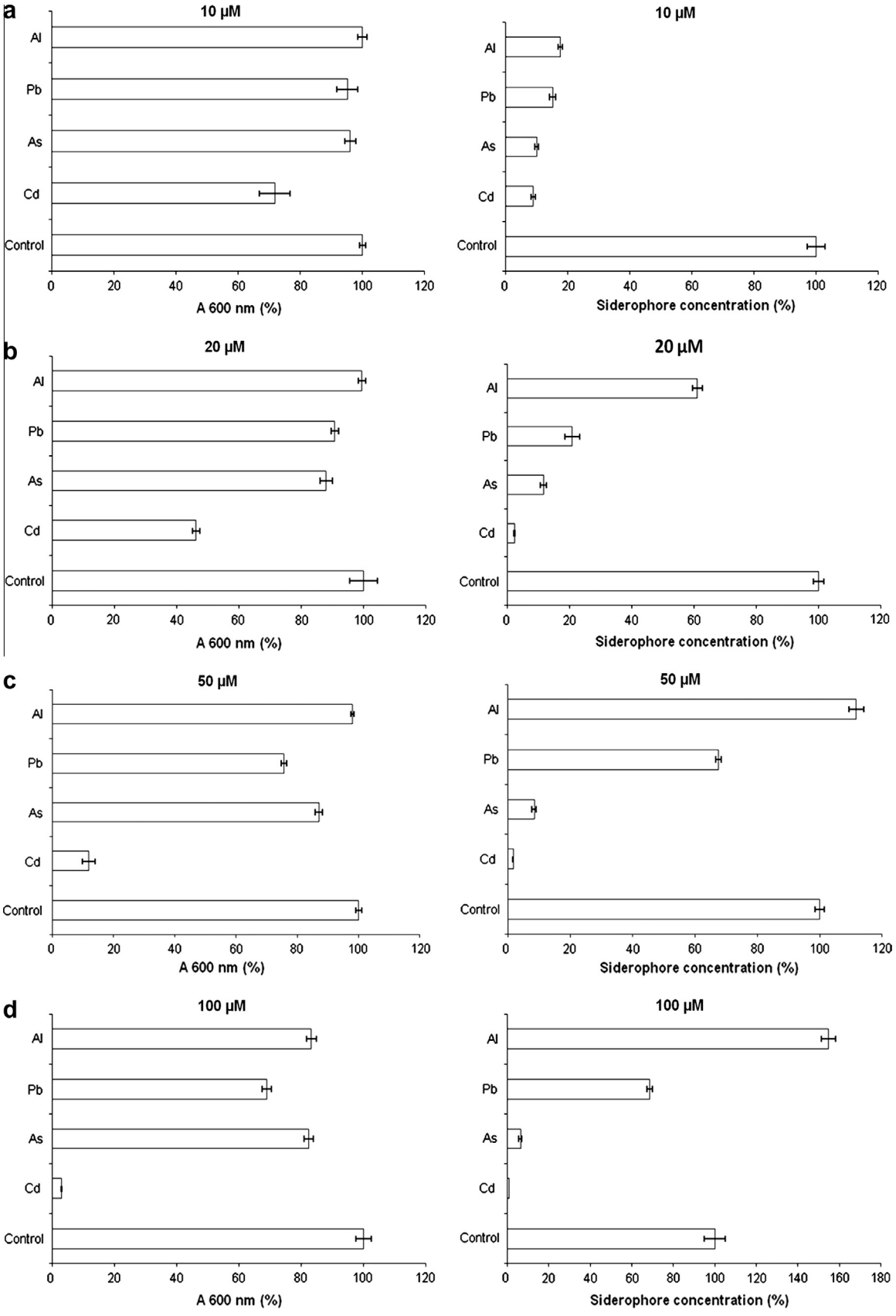

Fig. 6. Effect of Al, Pb, As and Cd on growth and siderophore production by *B. amyloliquefaciens* NAR38.1.

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trihydroxamates, desferrioxamine B (DFOB) and desferrioxamine E with the affinity which is near to or more than the affinity for Fe^{+3} (Duckworth et al., 2009). Molybdenum showed an increase in growth without any effect or increase in siderophore production (Fig. 4a–d).

Co showed a 40% and 60% decrease in growth at 10 (Fig. 5a) and 20 μM (Fig. 5b) respectively which was completely suppressed at higher concentrations of 50 (Fig. 5c) and 100 μM (Fig. 5d). Interestingly increase in the siderophore production in this culture was observed during growth at concentrations 10 and 20 μM with decrease at higher concentrations. Braud et al. (2009a,b) have also reported growth inhibition of *P. aeruginosa* with 100 μM of Co^{+2}.

With Cu, growth was decreased by 70% at 10 μM (Fig. 5a) beyond which it was completely repressed. However, siderophore production was increased by 20% at 10 μM Cu (Fig. 5a) and decreased at 20 μM (Fig. 5b) and above. Decrease in siderophore production with Cu has been reported by Arceneaux et al. (1984) who have shown decrease also in growth of *Bacilllus megaterium* when Cu was added at concentrations of 25 μM. Such a change is perhaps due to the accumulation of the Cu-siderophore (Schizokinen) complex resulting in a cellular accumulation of bactericidal levels of copper. Further, such high concentrations of copper in the cells are known to cause an oxidative stress eventually leading to DNA damage (Valko et al., 2005).

3.5.2. Effect of abiotic metals

One of the strategies for reducing the toxicity of the metal element is its binding to the siderophore. Amongst the abiotic metals, the most toxic element was found to be Cd, which inhibited the growth as well as siderophore production. An interesting observation was that Cd at low concentrations reduced the growth to 70% and 40% with 10 and 20 μM respectively (Fig. 6a and b respectively). However, siderophore production was inhibited to almost 90% at 10 μM (Fig. 6a) with negligible production at 20 μM (Fig. 6b). At higher concentrations of 50 and 100 μM, growth (Fig. 6c and d respectively) and siderophore production was reduced by more than 95% (Fig. 6c and d respectively). Cd has been reported to induce siderophore production in *Streptomyces* sp. (Dimkpa et al., 2008, 2009). The increase in siderophore production is a mechanism to alleviate the toxicity by binding free metal ions in the medium. Such a complex does not enter the cells and the toxicity is not manifested (Rajkumar et al., 2010). In the present study, Cd has decreased siderophore production due to inhibition of growth which implicates that such a mechanism does not exist in the isolate under study.

In the present study, it was noted that there was no significant decrease in the growth at different concentrations of Arsenic (Fig. 6a–d) however, there was no siderophore detected in the extracellular medium even at 10 μM of As (Fig. 6a). This implicates that the siderophore may be binding with As and is therefore not detectable with the test used for monitoring the siderophore. The implications of siderophore producing bacteria in metal contaminated soils with such a type of metal need to be examined for phytoremediation.

The effect of Pb on siderophore production was found to vary significantly with the concentration of the metal. Growth was found to reduce by almost 30% as the concentration was increased (Fig. 6a–d), however, the siderophore production increased with the increase in metal concentration showing almost 70% of production at 100 μM (Fig. 6d). Such an observation has also been reported with *P. aeruginosa* strain 4EA (Naik and Dubey, 2011). Siderophore producing organisms are also reported to reduce metal toxicity by binding to lead and prevent its diffusion into the cell (Schalk et al., 2011).

Studies with trivalent ion of Al did not show significant effect on the growth between 10 and 50 μM (Fig. 6a–c). A 20% decrease was however observed at 100 μM (Fig. 6d). Alternatively siderophore concentration in the supernatant was found to increase in the medium containing Al from 10 to 100 μM (Fig. 6a–d). The concentrations of 50 and 100 μM showed much better siderophore production as compared to the control.

Hu and Boyer (1996) have reported increase in siderophore (schizokinen) production from 65.7 to 140 μM in *B. megaterium* ATCC 19213 when the medium was supplemented with 370 μM of Al, with no effect on growth of the organism. A possible explanation for this may be that Al binds to the siderophore with the result that the cells do not acquire the required amount of iron, hence, to acquire iron for growth bacteria produce an increased level of siderophore. Al^{3+}, having similarity in size and charge as Fe^{3+}, has been shown to form complexes with hydroxamate type of siderophores which are also transported into the cell when Al is present at low concentrations (Garrison and Crumbliss, 1987; Hu and Boyer, 1996).

Production of siderophores in presence of metals can be a useful trait for plant growth promoting organisms as the soils which are contaminated with metals are often iron deficient (Tank and Saraf, 2009). In fact, siderophore producing bacteria have been considered important for inducing metal tolerance in plants and for the promotion of metal accumulation in plants. The effects of siderophore producing bacteria on the uptake of metals by hyperaccumulator plants have been the focus of increased attention (Dimkpa et al., 2008; Braud et al., 2009b). Braud et al. (2009b) have reported increase in the bioavailability of Cr and Pb in soils inoculated with *P. aeruginosa*. Bacterial siderophores can also provide iron to various plants, which helps in reducing the metal toxicity (Reid et al., 1986; Bar-Ness et al., 1991; Wang et al., 1993). Such beneficial effects exhibited by siderophore producing bacteria implicate that the inoculation with metal-resistant siderophore producing bacteria may help in improving the process of phytoextraction in metal contaminated soils.

4. Conclusion

Based on the studies, the effect of the metals on the isolate *B. amyloliquefaciens* NAR38.1, can be grouped into four different kinds of effect; one being increase in siderophore production as shown by Zn, Co and Mn. Zn did not have any effect on growth, while Co and Mn decreased siderophore production. Second being decrease in siderophore production with Mo and As without any effect on growth, the third was decrease in siderophore production at lower concentrations but increase at higher concentrations without affecting growth as manifested by Pb and Al. Cd and Co suppressed both, growth and siderophore production. The ability of this isolate to tolerate a range of pH and NaCl levels, and also produce siderophores in the presence of metals would prove to be useful in the restoration of the iron deficient soils contaminated with metals which stimulate siderophore production.

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