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Short Communication

Effect of Zerovalent Iron (Zvi) Nanoparticles on Siderophores Produced by Halophilic and Halotolerant Adhered Bacteria from the Mangrove Ecosystem

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Abstract

Iron being one of the most important elements required for metabolic activities of the bacteria has limited bioavailability. Therefore bacteria produce siderophores to sequester it from the environment. Halophilic and halotolerant adhered bacteria from the mangrove ecosystem were screened for the production of siderophores. Out of a total of 16 bacterial isolates 81% showed siderophore production. The effect of zerovalent iron (ZVI) nanoparticles was studied on the production of these siderophores. Among a total of 13 siderophore producing isolates 46.15% showed an increase in the siderophore production in the presence of ZVI nanoparticles. The ZVI affected growth negatively in 7% of the bacterial isolates.

Keywords: Zerovalent Iron; Nanoparticles; Siderophores; Mangroves; Adhered; Halophilic; Halotolerant

Abbreviations: DNA: Deoxyribonucleic Acid; NA: Nutrient Agar; NTYE: Nacl-Tryptone-Yeast Extract; CAS: Chrome Azurol Sulphonate; ZVI: Zerovalent Iron

Introduction

Mangrove ecosystem, faces a constant influx of tides and therefore halotolerant and halophilic bacteria predominate this ecosystem. It is one of the most efficient ecosystems of this planet [1] and for it to be efficient, the bacteria require many essential elements for their metabolic activities. Limitation in any essential element would mean a direct effect on the survival of the bacteria and the important role they play in nutrient recycling. One such essential element required for respiration, DNA synthesis and metabolic activities of bacteria is Iron (Fe). Though iron is the most abundant metal in the Earth's crust its bioavailability is limited, as owing to the aerobic atmosphere of the planet iron occurs mostly as ferric oxyhydroxide polymers which has low solubility iron. Therefore, due to the limited bioavailability of this metal bacteria have adopted strategies such as production of siderophores [2] Siderophores are iron binding compounds of weight less than 1000 Da that are produced by bacteria, fungi and plants. They chelate ferric ion from the environmental complexes and transport it to the bacterial cell [3,4,5].

Nanoparticles have been studied for their effect on the production of these siderophores in bacteria. Zinc oxide and copper oxide nanoparticles are reported to inhibit production of pyoverdine siderophore in *Pseudomonas chlororaphis* O6 [6]. Interestingly, a recent study reported the acquisition of iron from ferrihydride nanomineral by the bacteria *Pseudomonas mendocina* by siderophores and a cell-associated metalloreductase. This study also revealed the copious amounts of extracellular polymeric substances (EPS) produced by the *Pseudomonas mendocina* which enhanced the acquisition of iron from the nanomineral [7]. Zerovalent iron nanoparticles (ZVI) nanoparticles are being used for remediation of groundwater contaminated by perchloroethylene and trichloroethylene [8] and currently, ZVI nanoparticles are being studied for their role in bioremediation of other polychlorinated compounds [9,10,11] and uranium contaminated effluents [12]. Interestingly, iron nanoparticles represent the only field application of free released nanoparticles for environmental pollution therefore it was of interest to study their effect on mangrove bacteria.

In the present research work adhered halophilic and halotolerant bacteria that were isolated from the mangrove ecosystem were screened for the production of siderophores. The effect of ZVI nanoparticles on the production of these siderophores by the bacterial isolates was studied.

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Materials and Methods

5.1 Production of Siderophores by the Adhered Bacterial Isolates

Adhered bacterial isolates were isolated by the EDTA method [13]. These isolates were spot inoculated on Chrome Azurol sulphonate (CAS) agar with nutrient agar (NA or NaCl-tryptone-yeast extract (NTYE) as the base [14]. Negative control was maintained by addition of 0.1g/L of $FeCl_3$. The plates were incubated at room temperature for 24-48 hr and observed for zones of yellow colouration around the bacterial colonies.

Effect of Zerovalent Iron Nanoparticles (Zvi) on Siderophore

The selected isolates were spot inoculated on NA-CAS/ NTYE-CAS agar containing 0.1g/L of ZVI nanoparticles. CAS agar without ZVI nanoparticles served as the positive control for siderophore formation and CAS with 0.1g/L $FeCl_3$ was used as the negative control for siderophore. The plates were incubated at room temperature (28ºC) for 48-72 hr and the zones of colour change around the bacterial colonies were observed. The zone diameter was calculated by measuring the diameter of the zone divided by the diameter of the colony using a zone reader.

Results and Discussion

Screening of the Selected Bacterial Isolates for Production of Siderophores

Siderophores form stable complexes that are taken up by the bacterial cell and utilized for various metabolic activities. One of the isolates MXM-10 producing siderophores has been demonstrated in Figure 1.

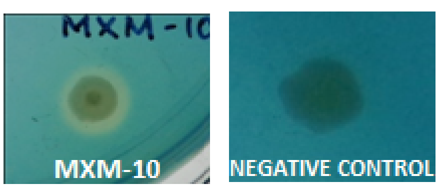

Fig.1: Isolate MXM-10 showing siderophore production on CAS agar

Among the 16 bacterial isolates that were screened, 81% showed siderophore production of which 62% were adhered halotolerant bacteria and 38% were adhered halophilic bacteria. It was observed that 19% of the isolates that did not produce siderophores were all halotolerant bacteria while all the halophilic bacteria showed the production of siderophores as shown in Figure 2. The high percentage of siderophore producers depict the iron deficiency for growth in the mangrove ecosystem which triggers the isolates to produce siderophores in order to obtain the element from the surrounding environment. Mangroves being one of the most efficient ecosystems, it was necessary to understand the ability of the bacterial isolates from this ecosystem to acquire iron and metabolize with optimum efficiency that contributes to efficient degradation and mineralization of particulate organic matter in this ecosystem. Earlier studies have shown siderophore producing *Pseudomonas* sp [15,16], *Escherichia coli, Bacillus subtilis* from coastal sand dunes [16] and Azotobacters from the tropical mangroves [17,18]. In the coastal and marine ecosystem, the iron concentration is low and in order to metabolise organic particulate matter containing aromatic ring bacteria use the enzymes oxygenases. These enzymes have iron as their cofactor and thus the ability of bacteria to produce siderophores is an important aspect in efficient degradation of particulate organic matter and pollutants such as aromatic anthropogenic compounds and hydrocarbons [16].

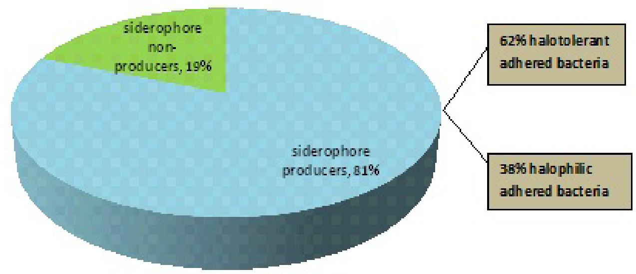

Fig. 2: Percentage distribution of siderophore producing halophilic and halotolerant adhered bacteria among the isolates.

Effect of ZVI Nanoparticles on Siderophore Production

Iron is the basic requirement for bacterial metabolism and its concentration in the surrounding environment has significant effects on cell processes and metabolic products. The effect of ZVI nanoparticles on siderophores was studied. Among the 13 siderophore producing adhered bacteria, 6 isolates showed an increase in the siderophore production in the presence of the ZVI nanoparticles (Figure 3).

A significant increase in the production of siderophores was observed in isolates MXM-1, MXM-10 and MXM -12. Similar increase in siderophore production has been studied in case of *Azotobacter vinelandii*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* in response to the presence of heavy metals such zinc cadmium, aluminium and nickel. Such stimulating effect on siderophores may be the result of the free siderophore concentration in the medium which decreases owing to the formation of siderophore-ion complex with ions other than Fe(III). This triggers an iron limitation and thus stimulates more siderophore production [17]. However in case of halotolerant isolate MXM-10 it was seen that a significant increase in the siderophore production, was also accompanied by a decrease in the diameter of the bacterial colony. Such negative effects on the growth of the bacteria due to the presence of metals have been reported by [19,20,21].

Conclusion

The halophilic and halotolerant adhered bacteria produced siderophores in order to overcome iron limitations in the mangrove ecosystem. The ability to produce such metal sequestering

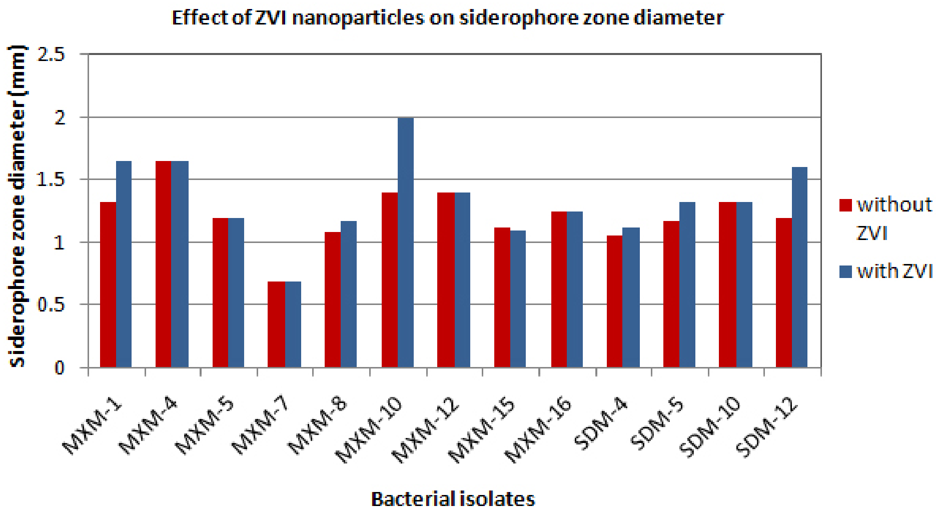

Fig. 3: The effect of ZVI nanoparticles on the siderophore zone diameter

Supplementary Material: Measurement of Zone diameter using zone reader

Isolate no	CAS		CAS+ZVI nanoparticles		CAS+ FeCl3	
	Mean Colony diameter	Mean Siderophore zone size	Mean Colony diameter	Mean Siderophore zone size	Colony diameter	Siderophore zone size
MXM-1	0.6	1.33	0.6	1.66	0.6	No zone
MXM-4	0.5	1.66	0.5	1.66	0.5	No zone
MXM-5	0.4	1.20	0.5	1.20	0.4	No zone
MXM-7	0.6	0.7	0.6	0.7	0.6	No zone
MXM-8	1.1	1.09	1.1	1.18	1.1	No zone
MXM-10	0.5	1.40	0.4	2.00	0.5	No zone
MXM-12	0.5	1.40	0.5	1.40	0.5	No zone
MXM- 15	0.8	1.12	0.9	1.10	0.8	No zone
MXM-16	0.4	1.25	0.4	1.25	0.4	No zone
SDM-4	1.6	1.06	1.6	1.13	1.6	No zone
SDM-5	0.6	1.17	0.6	1.33	0.6	No zone
SDM-10	0.6	1.33	0.6	1.33	0.6	No zone
SDM-12	0.5	1.20	0.5	1.60	0.5	No zone

compounds results in efficient degradation of particulate organic matter and nutrient recycling. The presence of ZVI nanoparticles like other heavy metals induced an increased production of siderophores. This may pave new pathways in strategies using siderophores in bioremediation of coastal and marine environments contaminated with heavy metals.

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