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Microbial Siderophore as a Potent Biocontrol Agent for Plant Pathogens

Zoology

KEYWORDS : Siderophore, antagonism, *Sclerotium rolfsii* and *Fusarium oxysporum*.

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ABSTRACT

Siderophores are compounds secreted under low iron stress, that act as a specific ferric iron chelate agents and due to their potentialities in the biological control of phytopathogenic fungi and bacteria their study have been stimulated in recent years. Siderophores produced by Pseudomonas species have been widely studied as biological agents and it is an alternative to take into account in the control of phytopathogenic microorganism in agriculture. In the present study, Pseudomonas fluorescens produced an extracellular siderophores when grown in Kings'B medium under iron deficiency. The optimal medium composition for the production of siderophore was 0.5 µM iron concentration, 55 µM glucose concentration, temperature 30°C, incubation time 72 hrs and pH 7.0. The UV spectral study of the produced siderophore showed maximum absorption peak ranged between 320 nm and 410 nm. The produced siderophores were antagonistic to fungal pathogens like Fusarium oxysporum and Sclerotium rolfsii. Hence Pseudomonas aeruginosa can be found efficient for siderophore production and biocontrol for fungal pathogens.

INTRODUCTION

The introduction of biotechnology products into agriculture has been improved in order to increase yields and crop quality, extremely important for developing countries (Cohen *et al*., 1998). Recently there has been an increasing interest in the use of biological control and siderophores produced by several of the fluorescent pseudomonas, as an alternative to take into account due to the fact that they reduce the rhizospheric population of phytopathogenic fungi and bacteria (Buysens *et al*., 1996; Fujimoto *et al*., 1995). Siderophores are thought to facilitate biocontrol by sequestering iron from pathogens, thus limiting their growth (Sullivan and Gara, 1992; Chiriani *et al.,* 1993; Champomier-veges *et al*., 1996). Siderophore production by strains of *Pseudomonas* spp., as a constituent of biological products, for plant disease control, is of great interest because its possibilities in the substitution of chemical pesticides (Loper and Lindow, 1987). Hence, the focus of the current research is detection, production, and optimization condition of siderophore by *Pseudomonas fluorescens* and its biocontrol efficacy.

MATERIALS AND METHODS

Bacterial culture and maintenance

Pseudomonas fluorescens, MTCC 665 strain used in the present experiment was collected from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India. *Pseudomonas fluorescens* strain was maintained on Pseudomonas phage agar medium.

Siderophore detection assay

For the detection of siderophore, *P. fluorescens* strain was grown in King's B liquid medium for 72 hrs on a rotary shaker at room temperature. The detection of siderophore assay was done by standard CAS plate method (Schwyn and Neiland, 1987).

Optimization of growth conditions for siderophore production

Once the detection of siderophore has been determined, growth conditions can be optimized in order to achieve the maximum amount of siderophore production. The optimization condition for siderophore production was done according to Clark *et al*. (2004). To test the effect of pH on siderophore production, culture inoculated flasks of King`s B medium at varying pH (5, 6, 7, 8, and 9) were incubated for 72 hrs. To optimize the temperature on siderophore production, culture inoculated flasks of King`s B medium at varying temperature (10, 20, 30, 40, and 50 °C) were incubated for 72 hrs. To determine the optimal incubation time on siderophore production, culture inoculated flasks of King`s B medium was incubated at varying time intervals (12, 24, 36, 48, 60, 72, 84, and 96 hrs). To optimize the glucose concentration on siderophore production, culture inoculated flasks of King`s B medium at varying concentration of glucose (40, 45, 50, 55, 60 and 65 µM) were incubated for 72 hrs. To test the optimal iron concentration on siderophore production, culture inoculated flasks of King`s B medium at varying concentration of iron (0, 0.5, 10, 15, 20 and 25 µM) were incubated for 72 hrs.

UV Spectral analysis

Ultraviolet (UV) spectral analysis of the produced siderophore was recorded on Shimadzu -1650 spectrophotometer. The spectra were recorded at 200-600 nm range.

Bioassay

According to De Villegas *et al*. (2002) 5 mm diameter mycelial disk taken from an actively growing colony of *Fusarium oxysporum* and *Sclerotium rolfsii* (grown on Sabouraud maltose agar) placed in the centre of a 9 cm diameter petridish containing Sabouraud maltose agar and 2 ml of sterile filtered supernatant of *Pseudomonas fluorescens*. Petridishes were incubated at 30°C and mycelial diameter was measured for 3 days. In the control plate, the sterile filtered supernatant was replaced by equal volume of sterile water. The inhibition of growth of fungal pathogens were recorded after 96 hrs of incubation and compared with the PDA plate inoculated with only pathogens as a control. The radial growth of mycelium was measured and per cent inhibition (PI) was calculated.

PI = A - T /A X 100

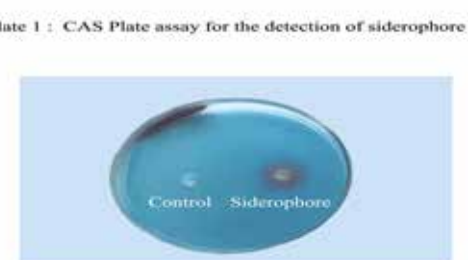
Where, A is the growth of test pathogen (cm) in the absence of the antagonistic strain, T is the growth of test pathogen (cm) in the presence of the antagonistic strain.

RESULTS

Siderophore detection

Fig-1 shows an orange halo around the well indicated that the presence of siderophores in the culture supernatant on CAS plates.

Fig- 1 CAS plate assay for the detection of siderophores



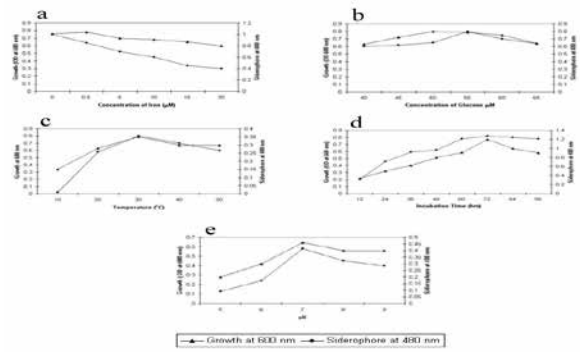
The bacterial strain used in the present experiment showed positive result in $FeCl_2$ test, CAS reagent assay and antagonistic

activity. It indicated that the experimental organism have the ability to produce siderophores.

Optimisation of growth conditions

The growth conditions need to be optimized for the maximum production of siderophore. The optimal medium composition for the production of siderophore was 0.5 µM iron concentration, 55 µM glucose concentration, temperature 30°C, incubation time 72 hrs and pH 7.0 (Figs-2a to 2e).

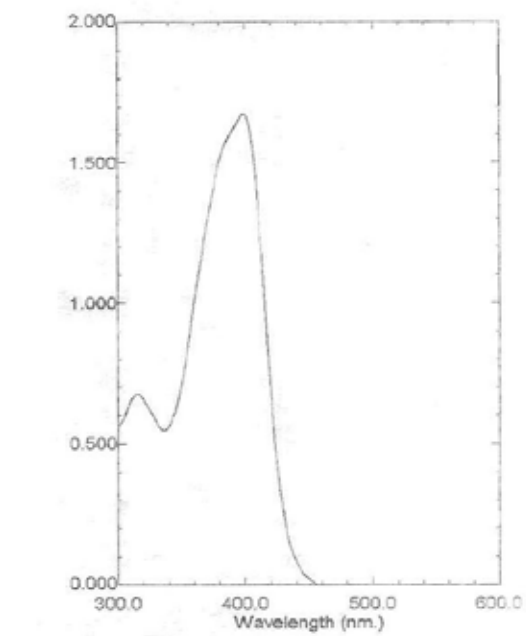
Fig- 2 Effect of iron concentration (a), glucose concentration (b), temperature (c), incubation time (d) and pH (e) on siderophore production



UV Spectral analysis

The spectral characteristic of the siderophore production by *Pseudomonas fluorescens* is given in Fig-3.

Fig- 3 UV-Spectral analysis of siderophore production by *Pseudomonas fluorescens*



It revealed that the cell free supernatant showed maximum absorption between 320 – 410 nm, which conferred the presence of siderophores. Ferric siderophore complexes showed absorption maxima between 405 – 420 nm.

Antifungal activity

The antifungal activity of *Pseudomonas fluorescens* strain showed antifungal activity against *Fusarium oxysporum* and *Sclerotium rolfsii* as target organism due to the production of siderophores (Fig-4). The 50 and 28.5% inhibition were recorded while using *Sclerotium rolfsii* and *Fusarium oxysporum* as indicator strains respectively.

Fig- 4 Antibiosis of *Pseudomonas fluorescens* against *Sclerotium rolfsii* (a) and *Fusarium oxysporum* (b)

DISCUSSION

Siderophores are compounds secreted under low iron stress, that act as a specific ferriciron chelate agents and due to their potentialities in the biological control of phytopathogenic bacteria their study have been stimulated in recent years. Clark *et al.* (2004) reported that initial detection of siderophore production was confirmed by using the CAS assay, which demonstrated the production of siderophore under iron-deficient conditions, with repression under high iron conditions. The color change from blue to orange resulting from siderophoral removal of Fe from the dye. Similar finding have been reported by Huston *et al.* (2004). Similary, Suryakala *et al*. (2004) investigated that production of siderophore was confirmed by positive ferric chloride test. In the present experiment, the produced siderophore was also Confirmed by positive ferricchloride test. Iron concentration of 10 µM is considered to be high and generally results in excellent cell growth with only modest yields of siderophores (Neilands, 1984). Manninen and Sandholm (1993) reported that siderophores production occurred only at an iron concentration of >50 µM and our results corroborate with this findings. Siderophore production was also studied at different pH. The maximum siderophore production was found at neutral pH 7.0 in which bacteria grow better and iron is present in insoluble form at neutral pH and therefore is not available to the bacteria (Sayyed *et al.,* 2005). The most surprising change in growth conditions was the increase in the incubation temperature from 27°C to 37°C, because the organism is a soil bacterium (Clark *et al.*, 2004). The antifungal metabolites inhibited the mycelial diameter of *Fusarium oxysporum* and *Sclerotium rolfsii.* Singh *et al.* (2006) found that *Pseudomonas* shows fungal growth inhibition by mechanism like antibiosis, site competition, HCN production, fluorescent pigments, antifungal volatiles metabolites and siderophore production.

CONCLUSION

Based on the data obtained in the present study it can be concluded that the major functional siderophore is generated by *Pseudomonas fluorescens*. The present study suggests that the produced siderophore may be used as potent biocontrol agent against plant pathogens and thus to create an ecofriendly environment in soil. Potential environmental applications of siderophores include recovery of toxic metals from industrial effluents and precious metals from mine. These results are promising in development of siderophore based Fluoresent pseudomonads as biocontrol agent.

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