International Journal of Advance Research in Science and Engineering
Vol. No.5, Issue No. 02, February 2016
www.ijarse.com
IJARSE
ISSN 2319 - 8354

STUDY Of EFFECTS OF DIFFERENT MEDIA ON THE PRODUCTION OF SIDEROPHORE BY CERTAIN MICROORGANISMS

Dr.Subhadra Rajpoot[1], Shivangi Asthana[2], Priyanka Bhati[3], Sachin Kumar[4]

[1,2,3,4]*Amity University Greater Noida (India)*

ABSTRACT

The production of siderophore by biocontrol agents (BGA) and plant growth promoting microbes (PGPM) is one of the important mechanisms for plant growth promotion and disease suppression. Microorganisms compete for iron by releasing siderophores. In this experiment, three fungi (Trichodermaviride-1 and T. harzianum-1 and Candida famata-1) and three bacteria (Bacillus subtilis-1, B. megatericus1, Pseudomonas aeroginosa1) are taken for their evaluation as siderophore producer by both qualitative and quantitative assay. All fungi and bacteria gave positive response to qualitative assay. In quantitative assay, among the fungi, C. famatagave maximum (60.00%) siderophore while among the bacteria and fungi, P. aeroginosayielded highest (80.50) percentage of siderophore. Moreover, effect of different media (MEB, NB, SMB, BRB and CCAB) on the siderophore production of P. aerogenosawas recorded where MEB supported maximum percentage of siderophore production (80.50%) but NB did not support. In modern science, production of pure siderophore in commercial way is very necessary as application of siderophore is in increasing trends in agriculture, medical science etc. Therefore, this work may be helpful for mass production of siderophore from microbes.

Keywords: Microorganism, Siderophore, Qualitative, Quantitative.

I. INTRODUCTION

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 conditions1. The production of siderophore by biocontrol agents (BGA) and plant growth promoting microbes (PGPM) is one of the important mechanisms for plant growth promotion2,3and disease suppression4-7. Siderophore producing bacteria have been used as biocontrol agents to combat plant pathogens8. Iron plays a central role in the energy metabolism of aerobic and semi-aerobic microorganisms9. Its availability in soil for microorganisms and plants drops dramatically with increasing pH above 6. The first report of a siderophore production was reported from *Ustilago sphaerogena*10. Then gradually, it was revealed that several fungi and bacteria are able to produce siderophores. Microorganisms compete for iron by releasing siderophores11. Typically, microbial sideophores are classified as catecholares, hydroxamates and α-carboxylates, depending on chemical nature of their coordination sites with

iron12,13. Some sideophores are as phenolates14 and others as mixed (both hydroxamate and catecholate functional groups)15. Pseudomonads generally produce fluorescent yellow-green and water soluble siderophoreswith both hydroxamate and phenolate groups; these siderophores have been classified as either pyoverdins or pseudobactins (Fig. 1). Iron competition in *Pseudomonads* has beenintensively studied and the role of the siderophore produced by *Pseudomonads* species were clearly demonstrated in the biological control of diseases16,7,17,10. *Pseudomonads* possess many traits that make them well suited as biocontrol and growth-promoting agents18. In addition, *pseudomonads* are responsible for the natural suppressiveness of some soils to soil borne pathogens19-21. Some fungi produce carboxylate while others produced hydroxamate type of siderophores. *Rhodotoulapilimanae*secreted rhodotorulic acid

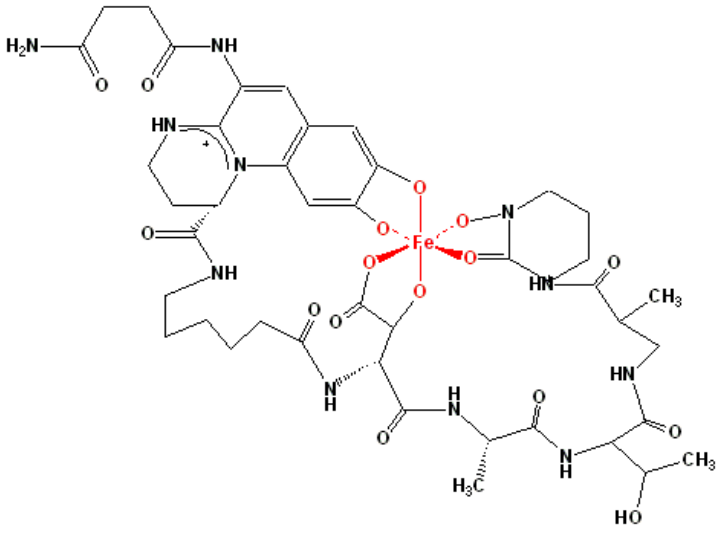

Fig. 1: Structure of Pseudobactin

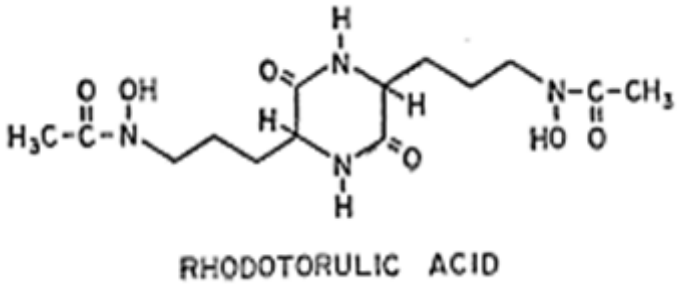

Fig. Structure of rhodotorulic acid

Recently, microbioal siderophores are isolated, purified and utilized, in addition to agriculture field2, in medical science for siderophore antibiotic preparation (Trojan horse antibiotics)24,25, in MRI (Magnetic Resonance Imaging) technique26 in cancer therapy27, as antimalaria28, antisleeping sickness29. The main objectives of this study were to screen some microbes for their ability of siderophore production, quantitative assay of it and effect of different media on its production.

II. EXPERIMENTAL

2.1 Material and methods

Detection in plate culture- In case of bacteria, universal Chrome Azurol S (CAS) agar medium was prepared as described by Schwyn and Neiland (1987)22 to detect the siderophore production. ME agar medium with ChromoAzurol S (CAS) (blue agar) was inoculated in the plate with 24 hr old bacteria and kept for incubation at 30oC for 72 hr. The blue colour of the medium to orange or presence of yellow to light orange halo surrounding the colony indicates the production of siderophore.

In case of fungi, the universal CAS assay was modified (CAS agar half plate30) to test the ability of fungal species to produce iron binding compounds of siderophore type in solid medium avoiding the growth inhibition caused by the toxicity of the CAS blue agar medium. Petri dishes (10 cm in diameter) were prepared with the MEA medium. After solidifying, the medium was cut into halves, one of which was replaced by CAS blue agar,the halves containing culture medium were inoculated with species taken from stock culture. The inoculums was placed as far as possible from the borderline between the two media, plates were incubated in the dark at 28oC for 6 days.

2.2 Quantitative estimation

MEB medium was prepared and used for siderophore production. 24 hr old culture of microorganisms were used to inoculate for 24 hr at 30oC with constant shaking at 120 r.p.m. Following the inoculation, fermented broth was centrifuged (10,000 r.p.m. for 15 min) and cell free supernatant was subjected to detection and estimation of siderophore. Quantitative estimation was done by CAS – Shuttle assays31,32. In which 0.5 mL of culture supernatant was mixed with 0.5 mL of CAS reagent and absorbance was measured at 630 nm against a reference consisting of 0.5 mL of uninoculated broth and 0.5 mL of CAS reagent. Siderophore content in the liquor were calculated by using following formula :

$$\% \text{ Siderophore units} = Ar–As/Ar \times 100 \ldots(1)$$

Where Ar = Absorbance of reference at 630 nm (CAS reagent)

As = Absorbance of sample at 630 nm.

2.3 Effect of different media

In case of effect of different media for production of siderophore, MEB (2% Malt Extract, pH 5.6)30, CAAB (containing g L-1 Cas-amino acid, 5.0; K2HPO4, 1.18; and MgSO4 7 H2O, 0.25 pH 5.6) 15, BRB (containing g L-1 K2HPO4, 0.1; KH2PO4, 3.0; MgSO4 7H2O, 0.2; (NH4)2 SO4, 1.0 and Succinic acid, 4.0, pH 5.6) (Barbhaiya and Rao 1985), SMB (consisting of g L-1 K2HPO4, 6.0; KH2PO4, 3.0; MgSO4 7H2O, 0.2; (NH4)2 SO4, 1.0; and Succinic acid, 4.0; pH 5.6)15 and Nutrient Broth (containing g L-1 peptone, 5.0; beef extract, 3.0; NaCl, 5.0;distilled water, 1L; pH 5.6) media were prepared and used forsiderophore production. 24 Hr. old culture of *Psedomonas aeruginosa*-1 was used to inoculate for 24 hr at 30oC with constant shaking at 120 r.p.m . Remaining procedure is same as earlier.

III. RESULTS AND DISCUSSION

The results presented in the Table 1 indicated that all bacteria and fungi tested gave positive response to siderophore production. Out of them *A. aeruginosa-1* indicated that it forms more yellow zone, then *B. subtilis*-1 and *C. famata*-1. On the other hand, fungal antagonist *T. viride*-1 and *T. harzianum*-I gave brown zone surrounding growth colony. It indicated that both fungi produced less siderophore in comparison to bacteria. In our experiment, *Trichodermaviride*and *T harzianum*indicated their positive response of siderophore production. It is at par the report of other workers33. The yeast *Candida famata*showed the ability of siderophore production. The secreted siderophores by this yeast were phenolate and hydroxymate type. Among the other yeast *Saccharomyces* sp and *Rhodotorula*sp gave 74.53% and 87.37% of siderophore, respectively33. Earlier, Schwn and Neiland22 reported *Rhodotoulapilimanae*secreted rhodotorulic acid siderophore.

Table 1: Detection of siderophore in CAS-MEA and modified CAS-MEA medium in plate culture

S.NO.	Antagonist	Colour of zone
1.	*T. viride*-1	+
2.	*T.harzianum*-1	+
3.	*P. aeruginosa*-1	++
4.	*B. megatericus*-1	+
5.	*B. subtilis*-1	+
+ = brown, ++ = yellow		

Among the bacteria tested in this study, all gave siderophore positive but *P. aeruginosa*gave more yellow zone than other bacteria and yeasts and fungi (Table 1). Siderophores are synthesized by many bacteria *Psedomonas*sp, *Azobacter*, *Bacillus*, *Enterobacter*, *Serratia*, *Staphylococcus* sp, *Azospirillum*and *Rhizobium*2,34,35,.

The results presented in Table 2 showed that *P. aeroginosa*-1 produced maximum percentage of siderophore (80.50) followed by *B. subtilis-1* (65.00), *C. famata*-1 (60.00), *Bmegatericus*-1 (50.00) and *T. harzianum*-1 (40.00) and *T. viride*-1 (30.00). Different organisms produced different percentage of siderophoresin their culture as reported by many authors29,33,3. Hussein and Joo (2012)33 reported that *T. harzianum*produced 92.33% of siderophore in MEB medium but in our study this *T. harzianum*-1 produced less amount and it was 40.00%. It may be due to different isolate of *T. harzianum*. Moreover, siderophore production depends on other factors such as iron content in medium, other minerals also influence its production. Zn2+ and Cu2+ increase florescent siderophoreproduction36. Cu2+ and Ni2+ also promote the production of yellow pigment or siderophore in *P. florescence –putida*37. Hussein and Joo33 also observed that *Aspergillusniger*produced 87.99% *Metarhiziumanisopliae*85.92% and *Penicilliumdigitatum*84.26% of siderophore in quantitative estimation.

.

Table 2: Quantitative estimation of siderophore produced by microorganisms

S.NO.	Antagonist	Siderophore %
1	*T. viride*-1	30.50e
2	*T. harzianum*-1	40.25d
3	*P. aeruginosa*-1	80.50a
4	*B. megatericus*-1	50.27c
5	*B. subtilis*-1	65.00b
6	*C. famata*-1	60.00b

The culture filtrate of *P. aeruginosa*-1 grown in five different media showing changes of colors (brown-yellow) were subjected to quantitative estimation of siderophoreproduction. Quantitative estimation of siderophore production of *P. aeruginosa*-1 in different media (Table 3) indicated that at pH 5.6 and at temperature 30oC, MEB medium exhibited maximum percentage of siderophore production unit (80.50%) followed by SMB (50.00%), BRB (40%) and CCAB (12%). Moreover, in NB medium, siderophore production by the *P. aeruginosa*-I was nil i.e. this bacterium cannot produce siderophore in NB medium.

IV. CONCLUSION

In conclusion, the tested fungi (*Trichodermaviride, T. harzianum and Candida famata*) all produce siderophore in qualitative test half CAS-ME agar medium andquantitative test CAS-ME broth media. These three fungi produce 30- 60% of siderophore inCAS-ME medium. All tested bacteria (P aeroginosa-1, B. subtilis 1, B megatericus 1)produce siderophore in qualitative CAS-ME media and qualitative CAS-ME broth.Moreover, they produce siderophore from 50-80.50% while *P aeroginosa*-1 is the bestproducer. Different media (MB, CCAB, BRB, MEB) supported siderophore production of*P. aeroginosa*1 except NB. Therefore, this study indicated the siderophore productionability by these microbes is in good amount, which are universally recognized biocontrolagents and plant growth promoting agents. Modern application of siderophore in agriculture,medical science and environment science are increasing. This study may help for moreproduction of siderophore in commercial way and more application of it in modern science.

REFERENCES

[1]. C. Ratledge and L. G. Dover, Annu. Rev. Microbiol., **54**, 881-941 (2000).

[2]. W. Kloepper, J. Leong, M. Teinize and M. N. Schroth, Nature, **286**, 885-886 (1980).

[3]. R. Z. Sayyed, M. D. Badgujar, H. M. Sonawane, M. M. Mhaske and S. B. Chincholkar, Indian J. Biotechnol., **4**, 484-490 (2005).

[4]. E. Husen, Indo. J. Agric. Sci., **4(1)**, 27-31 (2003).

[5]. C. Seuk, T. Paulita and R. Baker, J. Plant Pathol.,**4(3)**, 218-225 (1988). *S. K.* 1628 *Ghosh et al.: The Qualitative and Quantitative Assay of….*

[6]. S. Buysens, K. Heungens, J. Poppe and M. Hofte, Appl. Environ. Microbiol.,**62(3)**, 865-871 (1996).

[7]. Y. Eland and R. Baker, Ecol. Epidemiol., **75**, 1047-1052 (1985).

[8]. L. J. Gram, Microbiol. Meth.,**25(3)**, 199-205 (1996).

[9]. B. Schippers, Phil. Trans. R. Soc. Lond. B, **318**, 283-293 (1988).

[10]. J. B. Neilands, J. Am. Chem. Soc., **74**, 4846 (1952).

[11]. J. Leong, A. Rev. Phytopath., **24**, 187-209 (1986).

[12]. G. Winkelmann, Biotechnol. Adv., **8**, 207-231 (1991).

[13]. G. Winkelmann, Biochem. Soc. Trans., **30**, 691-695 (2002).

[14]. H. Haag, K. Hankte, H. Drechsel, I. Stojiljkovic, G. Jung and H. Zahner, J. Gen. Microbiol., **139**, 2159-2165 (1993).

[15]. J. M. Meyer and M. A. Abdallah, J. Gen. Microbiol, **107**, 319 (1978).

[16]. J. C. Beeker and R. Cook, J. Phytopathol., **74**, 806 (1984).

[17]. B. Sneh, M. Dupler, Y. Elad and R. Baker, J. Phytopathol., **74**, 1115-1124 (1984).

[18]. D. M. Weller, Annu. Rev. Phytopathol., **26**, 379-407 (1988).

[19]. D. M. Weller, J. M. Raaijmakers6, B. B. McSpadden Gardener and L. S. Thomashow, Annual Rev. Phytopathology., **40**, 309-348 (2002).

[20]. P. Banasco, Fuente L. De La, G. Gaultieri, F. Noya and A. Arias, Soil Biol. Biochem., **10 (10-11)**, 1317-1323 (1998).

[21]. J. Kraus and J. Loper, Appl. Environ. Microbiol.,**61(3)**, 849-854 (1995).

[22]. B. Schwyn and J. B. Neilands, Anal. Biochem.,**160**, 47-56 (1987).

[23]. B. P. Dave and H. C. Dube, Indian J. Exp. Biol., **38**, 56-62 (2000).