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PRODUCTION OF SIDEROPHORE (IRON CHELATOR) BY A.NIGER AND ITS ACTIVITY IN PLANT GROWTH

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ABSTRACT

*Micronutrient are those which needed by organism in less amount but play huge role in many cellular process and enzyme activity. Iron is one of the micronutrient which is universally required by all organism but as it is present in its insoluble form of Fe^{+3} state it is not utilized by organism so its conversion in Fe^{+2} state is necessary. Siderophore which is ferric specific iron chelator which chelates iron and make it available for plant in the form of ferrous iron. It is secreted by various fungi, bacteria and grasses in iron deficient condition. The complex of siderophore+micronutrient is absorbed by plant easily and then metabolized by again breaking the complex. One advantage of using siderophore instead of chemical fertilizer is that it's not poisonous and nor will it make any adverse effect on soil content. Instead it can also lessen the zinc deficiency in soilwith excess iron by binding to the iron and if iron is itself deficient then siderophore binds to iron and make it available to root of plant. For the production of siderophore**A.niger** is cultured in MEA media and SD-CASA method is used. Production of siderophore is determined by changing the colour of dye from blue to violet.*

Keyword: SD-CASA, A.NIGER, spectrophotometer, TLC, Bengal gram growth.

I. INTRODUCTION

siderophore is a iron chelating small molecular weight compound, secreted by bacteria, fungi, and grasses. It have high affinity for Fe^{+3} and transport it into bacterial cell. There are mainly three type of siderophore that are classified on the basis of chelating group specific for ferric iron they are catechol type, hydroxamate type and carboxyl ate type.

1) **Hydroxamatesiderophores** are produced by bacteria and fungi. Most hydroxamatesiderophores contain three secondary hydroxamate groups, C (=O) N-(OH)R, where R is an amino acid or a derivative. Each hydroxamate group provides two oxygen molecules, which form a bidentate ligand with iron. Therefore, each siderophore forms a hexadentate octahedral complex with Fe3+. Hydroxamatesiderophores usually show strong absorption between 425 and 500 nm when bound to iron. The hydroxamatesiderophores was detected by Neiland's spectrophotometric assay (1981) or by tetrazoliumsalt test (Snow, 1954).Hydroxamate type of siderophore is produced by ***A.niger***

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2)**Catacholatesiderophore** are also known as phenolatesiderophore. It is mainly produce by bacteria, and detected byNeilands spectrophotometric assay (Neilands, 1981),catecholate nature of siderophore is detected by formation of wine colouredcomplex with ferric chloride which show maximum absorption at 495nm, it can be confirmed by Arnows test by using nitrite molybdate reagent. Best example of this type of siderophore is Entrobactin produce by ***E.Coli.***

3) **Carboxylate type** of siderophore is produce by both bacteria and fungi. It contain both carboxyl and hydroxyl assay. This type of siderophore is produced by bacteria (***Staphylococcus*** strain and some ***Rhizobium***) and fungi which belongs to Mucorales.

Iron is most important micronutrient which is required in many cellular process like electron transport chain and in DNA synthesis. It also act as cofactor for many enzyme, such as succinic dehydrogenase, catalase, nitrogenase and peroxidase. Many nitrogen fixing microorganism are also produce siderophore hence at present days it is aimed to focus on siderophore mediated iron transport system. There are several method for detection of siderophore production but the most universal method is simple diffusion chrome azurolsulphate assay which is based on biological and function properties. This assay is based on colour changing properties in which when siderophore is produced by A.niger the blue colour of CAS dye get changes into violet or orange colour after chelation of the bounded iron by siderophore. (Schwyn and Neilands, 1987). This assay is based on the competition for iron between ferric complex of indicator dye and a siderophore produced by *A.niger*.

II. MATERIAL AND METHOD

1) Pure culture of *A. niger*NRRL 337. Stock cultures of fungal species were maintained on 2% malt extract agar (MEA) slant at 5°C.
2) Media:- MALT EXTRACT MEDIA (MEA MEDIA):- Suspend 18g agar powder in 1 lit of distilled water bring to boil to dissolve completely. Add 15 g malt extract per lit. Mix well and dispense as required and sterilize.
3) Preparation of glassware**:-**All glassware used was cleaned with 3 mol/L HCl to remove iron and rinsed in deionized water.
4) SD-CASA plate assay:-CAS-blue agar was prepared according to Schwyn and Neilands (Shin et al., 2001) using 0.06gm Chrome azurol S (CAS) dissolved in 50 mL distilled water from which 9ml solution is mixed with, 10 mL iron (III) solution (1 mmol/L FeCl3 .6H20, 10 mmol/L HCl). Under stirring, this solution was slowly added to 0.07gmHDTMA dissolved in 40 mL water. The resultant dark blue mixture was autoclaved at 121°C for 15 min. Agar (2%, w/v) was used as gelling agent. As CAS-blue agar medium is toxic hence it is modified to test the fungal species for production of siderophore in solid medium. For this petri dishes were prepared with MEA media. When it get solidified then halves of the plate medium cut and replaced by CAS-blue agar. The halves plate which contain MEA media were inoculated with spore of *A.niger*taken by stock culture. The inoculum were placed at some distance from borderline. After this incubate the plate at 28°C for 6 days. Fungi growth were measured per days and the CAS reaction was determined by measuring the position of colour change in CAS blue agar from borderline between two halves of the plate. CAS

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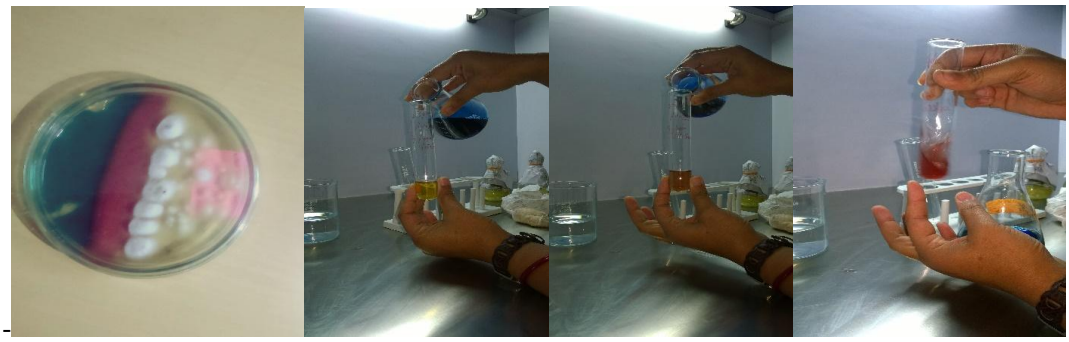
reaction rate is measured by making slope between distance of color change and number of days taken to cover that distance.

5) Fungal growth in liquid medium :- Fungal species were grown in 100 ml of 2% liquid maltextract, which were inoculated with one block of agar mycelium obtained from stock plates. Liquid malt extract media which contain iron (2 and 4 mmol l) were also inoculated with Aspergillus species. The flasks were incubated at 28°C for 6 days in stationary conditions. The broths were harvested, filtered through filter paper and taken for detection of siderophores. Results of siderophore production in liquid medium determined by CAS-liquid assay in which when CAS dye is poured slowly in filtered broth its color get change into violet color.
6) Siderophores detection in liquid medium:-A 3 ml supernatant of fungal liquid cultures was mixed with 3 ml CAS assay solution prepared according to Schwyn and Neilands (1987). A reference was prepared with exactly the same medium used for growing the fungi, but uninoculated. The sample (s) and reference (r) absorbanceswere measured at 630nm after 1 h of incubation at room temperature. The percentage of iron-binding compounds of the siderophore type were calculated by substracting the sample absorbance values from the reference. Siderophore units are defined as [(Ar) As/Ar)]100 = % siderophore units. Percentages of siderophores units less than 10 were considered as negative and in this case no change in the blue colour of CAS solution was observed.
7) Extraction of siderophore:-it is done by acetone precipitation method in this the culture supernatant(filtered) is mixed with ice chilled acetone in 1:3 and , Centrifuge at 10000 rpm for 15 min collect upper layer.
8) Comparative study of pot assay of Bangal gram:- The extracted siderophore was added in the soil in which Bengal gram was sown .In another pot , EDTA was added in the pot in which bangal gram was sown.$Fecl_3$ was also added in another pot.In one pot nothing was added which acts as control. Now growth werechecked, growth of plant in each pot are tabulate and growth percentage of siderophore were calculated.

III. RESULT



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The modified CAS-malt agar plates inoculated with *A.niger*showed many spores with hyphae. The plate showed change in colour in two days that was significant. So when fungal species is inoculated in broth medium and incubated at room temp for 6 days under stationary condition it produce siderophore which is tested by CAS shuttle assay in which when extracted broth is mixed with CAS dye it show change in color. Which confirmed that siderophore is produced by *A.niger*in malt extract broth. After spectrophotometric estimation and by performing Thin layer chromatography it is confirmed that A.niger produced hydroxamate type of siderophore. The extracted siderophore was now tested for growth of Bengal gram plant, which was also compared with EDTA, $Fecl_3$ and control. The pot in which siderophore was added showed faster growth than any other pot. The pot with EDTA showed growth intermediately. The control and pot with ferric chloride showed slower growth.

IV. DISCUSSION

Aspergillusniger produce more amount of siderophore which was confirmed by CAS-shuttle assay and it was confirmed that the above mention result were correct. Siderophore is iron chelating compound. In the pot assay the EDTA iron chelator was used to study the effect on plant growth. In this pot the plant growth was slower then the plant sown in the pot with siderophore in the control and the pot with ferric chloride growth was same and was slower than plant in the above mentioned pot. EDTA is not a natural iron chelating compound and hence this might be the reason that the plant growth was slower. Contrary to the siderophore is purely natural in origin and hence absorption in the cytoplasm might be higher than the EDTA iron complex.

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