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Isolation and screening of microorganism producing urate oxidase from poultry waste soil

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Abstract: Uricase (Urate oxidase) is an enzyme used to cure gout and tumor lysis syndrome, which is affiliated with chemotherapeutic treament of cancer. Urate Oxidase producing strains were isolated from soil. The culture is identified as *Aspergillus flavus* based on the morphological, microscopic and biochemical features. The ability of the culture growing on uric acid is clearly demonstrated by Disc Diffusion method and the presence to Urate oxidase gene is confirmed by molecular biology techniques.
Key words: Urate oxidase, Rasburicase, Gout, tumor lysis syndrome, *Aspergillus flavus* disc Diffusion method, PCR.

Introduction

Various enzymes were involved in the biosynthesis, interconversion, and degradation of purine compounds. Urate Oxidase is a liver enzyme which catalyzes the oxidation of Uric Acid to Allantoin in the presence of molecular oxygen. Urate Oxidase is a homo-tetrameric protein with four subunits with a molecular weight of the enzyme is 34.27 Kda[5]. *Aspergillus flavus* is the good source of Urate Oxidase enzyme.

The end product of purine metabolism varies among various species (Figure 1.1.1) due to loss of function in the respective gene during evolution. Uric acid is the end product of purine metabolism in humans, hominiods, birds and reptiles[6]. Allantoin is the end product of purine metabolism in mammals other than primates[1]. In amphibians and fishes, allantoin is converted to urea with the help of allantoinase enzyme[2]. Plants and microorganism have to ability to metabolise purines into Ammonia and Carbon dioxide[7]. The enzyme is functional in many organisms but absent in humans. A gene for Urate Oxidase is found in humans but it is non-functional due to nonsense mutations in the gene[4]. But in turn, bacteria and yeasts break down uric acid into Allantoin with the help of Urate Oxidase enzyme[3]. The therapeutic demand of the enzyme has created a space to isolate and screen Urate Oxidase producing microorgansim. A simple method to isolate and screen the microorganism producing urate oxidase is exemplified in this research paper.

Materials and Methods:

Chemicals, reagents and enzymes:

Molecular biology chemicals, enzymes and kits were purchased from Fermentas, Sigma Aldrich and Qiagen. Glassware and other purification chemicals were of analytical grade purchased from local companies.

Isolation of Urate Oxidase producing microbe*:*

About 10 g of the soil sample is aseptically collected into a plastic bag. One gram of the soil was weighed and suspended in 9 ml of sterile distilled water and is properly mixed. 1ml of the soil suspension is

pipetted from each of the above and transferred into another 9 ml of sterile distilled water. The soil suspension is further serially diluted in seven tubes. About 0.2 ml from the each dilution was spread on nutrient agar and potato dextrose agar plates using a glass spreader which is sterilized by 95% ethanol. The plates were incubated at room temperature for 5 days for fungal isolates and overnight incubation at 37^0C for bacterial isolates. The growth of fungal and bacterial colonies were observed after incubation. The individual colonies were subcultured and preserved. Identification of the culture was based on cell and colony morphology characteristics (morphological and microscopic features). The characteristics used are colonial characteristics such as size, surface appearance, texture and colour of the colonies.

Screening of Urate Oxidase producing microbe:

Isolated individual colonies were streaked on the screening medium and incubated at 25^0C for 48-72 hours. At the end of incubation period, cultures grown on the selective medium were subcultured and preserved.

C**onfirmation of culture by disc diffusion method:**

Isolated individual colonies was grown in Potato Dextrose broth medium (pH 6.0) at 25^0C. For sub-culturing, about 5.0 %v/v of inoculum was inoculated into sterile 25ml Potato Dextrose broth medium (pH 6.0) in a conical flask. The inoculated Potato Dextrose broth was incubated at 25^0C for 48 hours. At the end of incubation period, 50 µl of microbial culture was applied on a sterile disc and placed in a Potato Dextrose agar medium containing 0.3 % w/v uric acid. Sterilized Potato Dextrose broth medium was used as a control. The plate was incubated at 25^0C for duration of 24-48 hours. Zone of clearance around the well formed in the solidified medium.

Growing of *Aspergillus flavus*:

The fungal spore inoculum was prepared by adding 10 mL of the sterile distilled water containing Tween 80 to the PDA slants where culture was maintained. The spores were freed using a sterile inoculation loop under aseptic conditions. About 1 ml of spore suspension was used as the inoculum. The inoculum was added into a medium containing 1 g of KH_2PO_4, 2 g of NH_4NO_3, 0.2g of $MgSO_4.7H_2O$, 20 mg of $CaCl_2.2H_2O$, 4 mg of $MnSO_4$, 2 mg of Na_2 $MoO_4.2H_2O$, 2.5 mg of $FeSO_4$. $7H_2O$, 20 g of Uric acid and made up to 1 liter with water and the pH was adjusted to 5.5.The spore suspension was inoculated into 250 mL conical flask containing 100 mL of the above media. The culture was grown for 96 hours at 35°C in a shaking incubator. Small ball to filamentous structures were observed after 96 hours.

C**onfirmation of culture by PCR method*:***

The 96 hours grown culture was centrifuged at 10000 g for 20 min at 4ºC. 100 mg of mycelia mat was used as a source of material for purity total RNA. Total RNA was isolated using RNeasy Kit as per the technical data sheet provided by Qiagen. Omniscript RT kit (Qiagen) was used for the synthesis of cDNA. The synthesized cDNA was used as a template to synthesize Urate Oxidase gene by using Urate Oxidase specific primers. The parameters used in the reaction were, an initial denaturation for 4 minutes at 94^0C, followed by 25 cycles of denaturation for 20 seconds at 94^0C, annealing for 30 seconds at 48^0C, extension for 20 seconds at 72^0C and final extension at 72^0C for 4 minutes.

Results and Discussion:

Isolation and screening of Urate Oxidase producing microbe:

Urate Oxidase producing strains were isolated from soil. The growth of fungal and bacterial colonies was observed (Figure 1). The individual colonies were subcultured and preserved. Out of 22 cultures isolated, the best urate oxidase producer was selected and pure culture was done. Identification of culture was based on cell and colony morphology characteristics (morphological and microscopic features). The culture was identified as *Aspergillus flavus* based on colonial characteristics such as size, surface appearance, texture and colour of the colonies (Figure 2).In the Urate Oxidase Disc Diffusion method, a clear zone of clearance was formed around the disc with the culture of *A.flavus* and no clearance was observed around the disc with the media. This clearly reveals that the selected culture is capable of utilizing uric acid for their growth (Figure 3).

Figure 1: Plate showing the colonies capable of growing on uric acid containing medium,

Figure 2: Culture of *Aspergillus flavus*

Figure 3: Urate Oxidase Disc Diffusion assay
C:Control; S:Sample

Confirmation of culture by PCR method*:*

The PCR product electrophoresed on 1% Agarose gel showed the migration of UOX gene at 900 base pairs indicating the presence of the Urate oxidase gene (Figure 4).

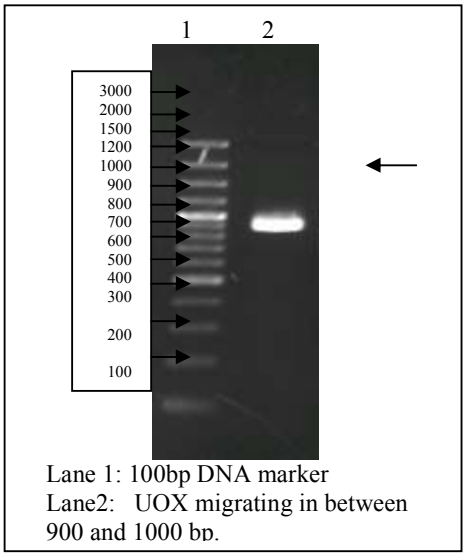

Figure 4: Agarose gel showing the amplified DNA

Conclusion:

The genus *Aspergillus* obtained its name from Micheli in 1729. Asexual spore head that looks like an aspergillum are the characteristic of the genus *Aspergillus* (Geiser *et al.,* 2008). They are of high pathological, agricultural, industrial, pharmaceutical, scientific, clinical importance[8,9,10]. Many *Aspergillus* species and strains have industrial importance like the production of extracellular enzymes, organic acids and secondary metabolites[8].

Aspergillus flavus lives in soil and plays an important role in element cycling by using dead organic material as nutrient. *Aspergillus flavus* produces large number of extracellular hydrolytic enzymes to breakdown complex materials for their growth.

Urate Oxidase producing strains were isolated from soil. The culture is identified as *Aspergillus flavus* based on the morphological, microscopic and biochemical features. A simple method to isolate and screen the microorganism producing urate oxidase is illustrated. The ability of the culture growing on Uric acid is clearly demonstrated by disc Diffusion method and the presence to Urate oxidase gene is confirmed by PCR method.

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