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# *In Vitro*, Optimization of Antibacterial Activity of Secondary Metabolites Produced By *Aspergillus Flavus* (Mtcc-3396)

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## ABSTRACT

The purpose of the present study was to investigate the influence of cultural conditions and environmental parameters affecting the growth and bioactive metabolite production of the fungi ***Aspergillus flavus*** (MTTC-3396) which exhibits a broad spectrum of in vitro antibacterial activity against human infecting bacterial pathogens and the high bioactive metabolites production. The effect of various parameters viz., incubation time, temperature, pH, carbon and nitrogen sources, and sodium chloride concentration on antibacterial metabolite production were studied by varying single parameter at a time. It was found that metabolite production by this isolate was greatly influenced by various cultural conditions. The optimum carbon source starch, nitrogen source beef extract, pH 6.0, temperature at 40$^{o}$C, 3 % NaCl concentration and incubation period of 144 h were found for the maximum metabolite production.
**Keywords:** ***Aspergillus flavus*** (MTCC-3396), Metabolite production, Optimization.

## I. INTRODUCTION

Microbial secondary metabolites have provided numerous pharmaceutical agents ranging from antibiotics to immunosuppressive compounds. Synthesis of these low molecular weight compounds is not required for normal growth of the microbe; however these compounds may provide several benefits to the organism. Fungi have the ability to produce a plethora of secondary metabolites, typically dependent on the stage of development and environmental factors ranging from nutrient concentrations to light and temperature (**Calvo et al., 2002; Keller et al., 2005**). Fungi are considered as a good natural source for a production of bioactive secondary metabolites that contain different bioactive agents including antibiotics, anti-tumors, and antioxidants (**Elaasser et al., 2011**). Endo-phytes are microbes that colonize living, internal tissues of plants without causing any harmful, overt negative effects (**Bacon et al., 2000**).

Since microorganisms grow in unique and extreme habitats, they may have the capability to produce unique and unusual metabolites. Generally, the reason why they produce such metabolites is not known, but it is believed that many of these metabolites may act as chemical defense as an adaptation of fungi competing for substrates (**Gallo et al.**,) The antimicrobial properties of secondary metabolites derived from various groups of fungi are widely reported (**Sekiguchi and Gaucher, 1977; John et al., 1999; Schulz et al., 2002; Keller et al., 2005**), suggesting the outstanding potentiality of this microbial community as an important source of bioactive molecules. *Aspergillus*, a fungi represented by large number of species, is known to produce anti-*hilicobacter pylori* secondary metabolites like helvolic acid, monomethylsulochrin, ergosterol and 3β-hydroxy-5ά (**Gao et al., 2007**) and cytotoxin, Brefeldin A (**Wang et al., 2002**). There have been several studies on the antimicrobial potentiality of *Aspergillus* spp. against a panel of bacterial and fungal pathogen (**Maria et al., 2005; Kumar et al., 2010**). Antimicrobial activities of an endophyte *Aspergillus* sp. against some clinically significant human pathogens have been reported (**Tayung and Jha, 2007**). Ability of antagonistic fungal strains to produce bioactive metabolites.

## II. METHODS AND MATERIAL

**Culture Collection and Maintenance:**

Pure cultures of ***Aspergillus flavus*** (MTCC-3396) were purchased from MTCC, Chandigarh, India and were immediately transferred to sterile agar slants of potato dextrose agar media. The strains were grown in potato dextrose media. The *Asperigillus sps.*, culture from potato dextrose broth was streaked on a Potato dextrose agar slant and it was incubated at 27$^{o}$C for 72 hours. It was then sub cultured and was stored in refrigerator for further use.

**Microbial Target Organisms**

The organisms like ***Staphylococcus aureus***(MTCC-3160),***Streptococcus***(MTCC-2327), ***Ksebsiella pneumoniae*** (MTCC-452), ***Escherichia coli (***MTCC-443), ***Bacillus coagulans*** (MTCC-), ***Bacillus subtilis*** (MTCC-441), ***Corynibacterium glutamicum*** (MTCC-2745-), ***Spinghomonas*** (MTCC-6363) was procured from Microbial Type Culture Collection(MTCC) and Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh, India and maintained freshly prepared potato dextrose agar slants respectively.The organisms were preserved at - 20 ºC in the presence of glycerol (15 %, v/v) for longer periods.

**Basal Medium:**

Potato dextrose broth medium was used as a basal medium. Twenty five milliliters of the medium dispersed in 150 mL conical flasks and sterilized. The fungal culture were inoculated with 5 mm diameter, mycelial disk obtained from 7day old spore culture of *Aspergillus sps.*,and incubated at 28°C for 14 days. After incubation the growth of the isolate was determined as dry mycelial weight in 25 mL of culture medium. The mycelia were harvested by filtration using whatman filter. Then the mycelia were washed thoroughly with distilled water and the excess of water removed by blotting with filter papers. The mycelia were then allowed to dry at 80°C and expressed as dry weight of mycelia (mg/25 mL). The production of bioactive metabolites was expressed by measuring the diameter of the inhibition zone against test organisms including ***Escherichia coli, Staphylococcus aureus Klebsiella pneumonia, Streptococcus, Spinghomonas, Bacillus coagulans***

**Selection of the Culture Medium**

To select the suitable growth medium for the production of secondary metabolites. The isolate was grown in different culture media like Sabarouds dextrose broth, Nutrient broth, Malt extract broth, Czapeck dox broth, Sabarouds glucose broth. For growth and secondary metabolite production, in which medium the isolate exhibit maximum secondary metabolite production was used as the optimized medium for further study. All the media were procured from HiMedia Laboratories, Mumbai, India.

**Effect of Temperature on growth and secondary metabolite production:**

The effect of incubation period on growth and secondary metabolite production was investigated by incubating the fermentation medium at regular intervals of 72hr to 240 hr at pH 5.6 and at 350C. After the incubation period biomass (mycelial dry weight) and the production of bioactive metabolites were recorded at the end of incubation periods

**Effect of Temperature on growth and secondary metabolite production:**

The effect of temperature on growth and secondary metabolite production was investigated by incubating the fermentation medium at 20 to 45°C at pH 6 for 240hrs. After the incubation period biomass (mycelial dry weight) and the production of bioactive metabolites were recorded at the end of incubation periods

**Effect of NaCl, Carbon and nitrogen sources on growth and secondary metabolite production:**

The effect of different Nitrogen sources on growth and secondary metabolite production was investigated by adding carbon sources sucrose, maltose, lactose, fructose, starch, D-mannitol, nitrogen sources like soybean meal, yeast extract, beef extract, sodium nitrite, potassium nitrate, ammonium sulfate and NaCl concentrations (3-9%) separately to the fermentation medium and incubating them at pH 6 for 144hrs at room temperature. After the incubation period biomass (mycelial dry weight) and the production of bioactive metabolites were recorded at the end of incubation periods.

**Effect of Minerals on growth and secondary metabolite production:**

The effect of different mineral sources on growth and secondary metabolite production was investigated by adding Mineral sources $ZnCl_2$, KCL, $CuCl_2$, $CoCl_2$, $MnCl_2$, $MgCl_2$ separately to the fermentation medium and incubating them at pH 6 for 144hrs at room temperature. After the incubation period biomass (mycelial dry weight) and the production of bioactive metabolites were recorded at the end of incubation periods.

## III. RESULTS AND DISCUSSION

### RESULTS

The yield of bioactive compounds can sometimes be substantially increased by the optimization of physical (temperature, salinity, pH and light) and chemical factors (media components, precursors, and inhibitors) for the growth of microbes

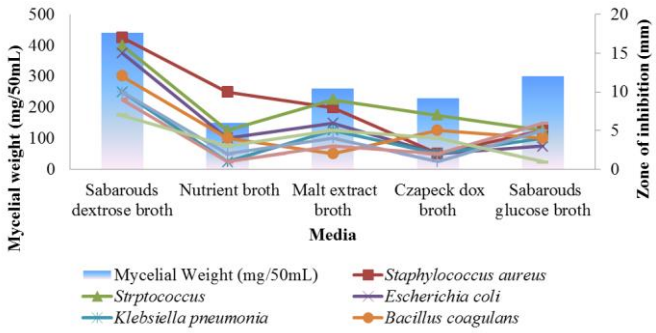


**Figure 1.** Effect of media on growth and antibacterial metabolite production in *Aspergillus flavus*

In Fig-1- revealed that effect of different media on growth and antibacterial metabolite production in ***Aspergillus flavus***. Among the tested media maximum mycelia weight (440mg/50ml) observed in sabrouds dextrose broth, minimum mycelia weight (150mg/50ml) in nutrient broth and maximum antibacterial metabolite production produced in sabrouds dextrose broth(15mm against ***Staphylococcus aureus)***.

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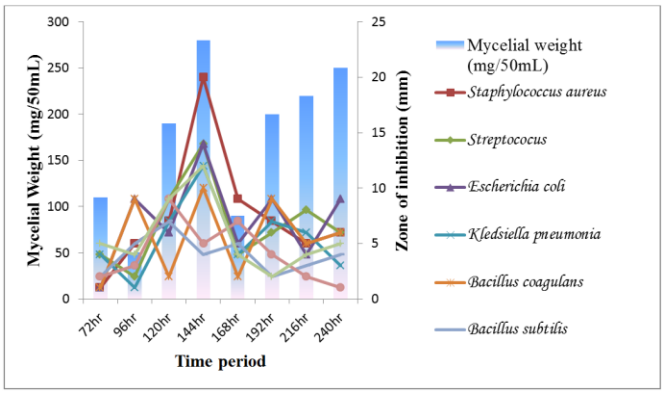


**Figure 2.** Effect of Time period on growth and antibacterial metabolite production in *Aspergillus flavus*

In Fig-2- revealed that effect of different Time period on growth and antibacterial metabolite production in ***Aspergillus flavus***. Among the tested time periods maximum mycelia weight (290mg/50ml) observed in 144hr and maximum antibacterial metabolite production produced in sabrouds dextrose broth(20mm against ***Staphylococcus aureus)*** at pH 6

In Fig-3- revealed that effect of different Temperature on growth and antibacterial metabolite production in ***Aspergillus flavus***. Among the various temperature ranges maximum mycelia weight (120mg/50ml) observed and maximum antibacterial metabolite production produced in at $30^0$C (11mm against ***Escherichia coli)*** at $30^o$C for 144hr of incubation period at pH-6

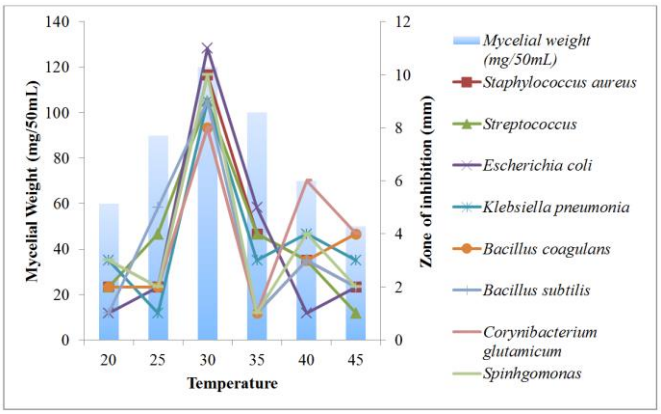


**Figure 3.** Effect of Temperature on growth and antibacterial metabolite production in *Aspergillus flavus*

In Fig-4- and Fig-5- revealed that effect of different carbon and nitrogen sources on growth and antibacterial metabolite production in ***Aspergillus flavus***. Among the various carbon nitrogen sources starch and beef extract is the best carbon, nitrogen sources for maximum mycelia weight (700mg/50ml), (540mg/50ml) observed and maximum antibacterial metabolite production produced in fructose and beef extract (10mm against ***Bacillus subtilitus***, ***Spinghomonas***), (15mm against *Escherichia coli)* at 30$^{o}$C for 144hr of incubation at pH-6

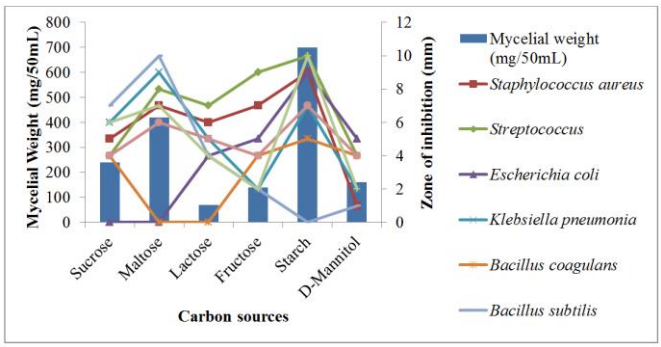


**Figure 4.** Effect of Carbon source on growth and antibacterial metabolite production in *Aspergillus flavus*

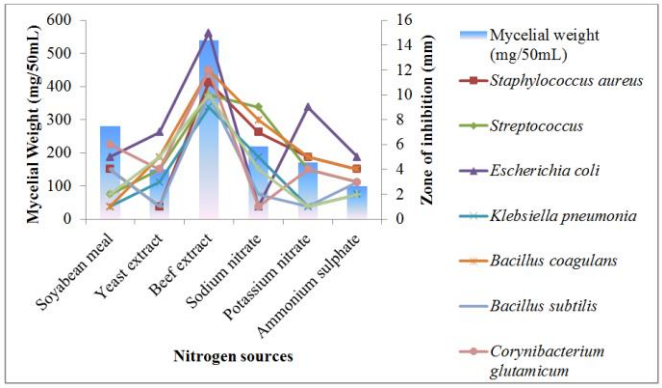


**Figure 5.** Effect of Nitrogen sources on growth and antibacterial metabolite production in *Aspergillus flavus*

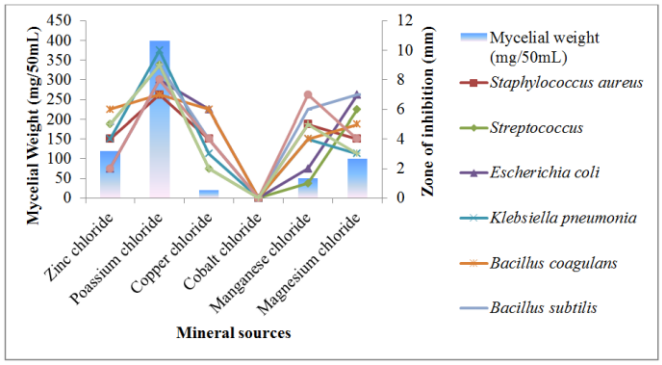


**Figure 6.** Effect of Mineral sources on growth and antibacterial metabolite production in *Aspergillus flavus*

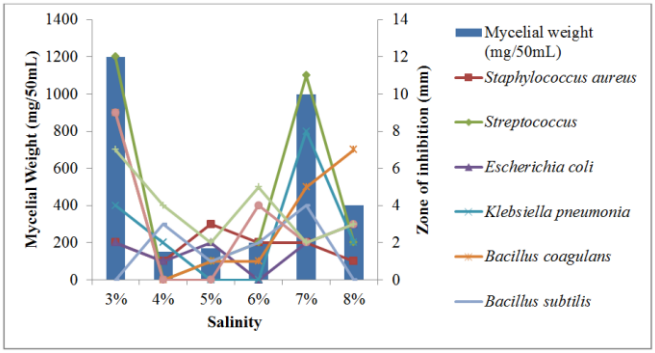


**Figure 7.** Effect of Salinity on growth and antibacterial metabolite production in *Aspergillus flavus*

In Fig-6- and Fig-7- revealed that effect of different mineral source and salinity concentration on growth and antibacterial metabolite production in *Aspergillus flavus*. Among the various mineral sources potassium chloride is the best mineral sources for maximum mycelia weight (400mg/50ml), observed and maximum antibacterial metabolite production produced in potassium chloride (10mm against *Klebsiella pneumoniae*) and NaCl concentration of 3g/100ml was recorded as optimal for maximum mycelia weight (1200mg/50ml) and improved antibacterial metabolite production (11mm against *Streptococcus*) at 30$^{o}$C for 144hr of incubation at pH-6

## DISCUSSION

For the past five decades, the need for new antibiotics has been met largely by semisynthetic tailoring of natural product scaffolds discovered in the middle of the 20$^{th}$ century. More recently, however, advances in technology have sparked a resurgence in the discovery of natural product antibiotics from microbial sources. In particular, efforts have refocused on finding new antibiotics from old sources (for example, streptomycetes) and new sources (for example, other actinomycetes, cyanobacteria, uncultured bacteria and fungi). This has resulted in several newly discovered antibiotics with unique scaffolds and/or novel mechanisms of action, with the potential to form a basis for new antibiotic classes addressing bacterial targets that are currently underexploited. Natural products represent the traditional source of new drug candidates. Maximum growth and metabolite production observed on sabarouds dextrose broth at 30$^{o}$C for 144hr of incubation. Similarly, the effect of culture medium on mycelial growth, metabolite profile and antimicrobial compound yield by a marine-derived fungus *Arthrinium*

c.f. *saccharicola* was investigated by Miao et al., (2006), suggesting the need of optimal culture composition to achieve maximal mycelial growth and bioactivity of the fungus. Different studies proved that temperature is one of the major conditions affecting the growth rate of antagonist (Kok and Papert, 2002) values gradually decreased with increase in salt concentration in the basal media. NaCl concentration of 3.0 % was found to be optimum for maximum growth (1200mg/50ml) and production of bioactive metabolite (10.6 lg/ml, 10.1 lg/ml) by an antagonist fungus, *Fusarium* sp. (Gogoi et al., 2008). The present work revealed that 3% of NaCl concentration was found to be optimum for maximum antibacterial metabolite production. Starch was the least utilized carbon compound by the isolate and even the bioactive production was very low. Bhattacharyya and Jha. (2011) reported that the *Aspergillus* sp. grew on all the carbon sources and tested against bacterial pathogen *Bacillus subtilis*, and the maximum growth and bioactivity of the strain was noted when the sucrose was used as a sole carbon source. The results are in good agreement with Thakur *et al.,* (2009) fructose is the best source for maximum growth and antibacterial metabolite production. Maximum biomass production (600 mg/50mL) and antibacterial activity (20 mm zone of inhibition against *Escherichia coli* ) was observed in culture filtrate supplemented with yeast extract. Peighamy-Ashnaei *et al.,* (2007) have described the importance of various nitrogen sources in maximizing the growth rate of the fungal strain and the antibiotic production.

## IV. CONCLUSION

In the present study, concluded that the optimum conditions required for the production of bioactive metabolite by seaweed fungi **Aspergillus flavus** were determined and metabolites showed better antimicrobial activity against human pathogens. Hence the further studies carried on purification, characterization and identification of bioactive metabolites of ***Aspergillus flavus*** MTCC-3396.

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