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Optimization and antimicrobial metabolite production from endophytic fungi *Aspergillus terreus* KC 582297

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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 seaweed endophytic fungi Aspergillus terreus KC 582297 which exhibits a broad spectrum of in vitro antimicrobial activity against human infecting bacterial pathogens and the high bioactive metabolites production was observed in potato dextrose broth, compared to the other media. Glucose and yeast extract were found to be a best and most suitable carbon and nitrogen sources respectively, for the optimum growth and production of bioactive metabolites. Maximum bioactive metabolite productions occur in pH of 5.5 and temperature at 25°C.

Keywords: *Aspergillus terreus*, bioactive metabolite production, optimization, endophytic fungi.

INTRODUCTION

Endophytic fungi are found in all divisions of fungi and most probably evolved the association independently on many occasions. The most common endophytes are anamorphic members of the Ascomycota, and some are closely related to fungi known to cause disease in plants and animals. Marine derived microbes especially fungi have long been recognized as a potential source of novel and biologically potent metabolites Saleem *et al.*, (2007). Many of the microbes live in extreme environments such as high temperatures, high salt concentrations, low pH, and high radiation. Some of the physical factors also influence the fungal growth and metabolite productions. Usually the biotechnological production from microorganisms based on their special adaptations to their environment Padmavathi *et al.*, (2012). Some of primary metabolism serves as a branching point of biosynthetic pathways which leads to the end product of primary and secondary metabolism. Growth media and incubation conditions have a very strong influence of secondary metabolite production. There is no consensus on which media are the optimal for metabolite production. Secondary metabolism is regulated by carbon sources, nitrogen sources, phosphate, trace elements, precursors, induction of enzymes of secondary metabolism, catabolic repression and inhibition, feedback repression and inhibition and it controlled by auto-regulators Betina, (1994). Natural environment is still the most important contributor of novel drugs in the face of development of combinatorial chemistry, which quickly generated thousands of new chemicals Zhang *et al.*, (2010). The pure culture of *Aspergillus terreus* isolated from seaweed *Codium decorticatum* maintained in potato dextrose agar medium at 4°C was used. Further studies were carried out to optimize the culture conditions of the isolate *Aspergillus terreus* to enhance the growth and production of biological active compounds.

MATERIALS AND METHODS

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 species were inoculated with 5 mm diameter, mycelial disk obtained from 7day old spore culture of *Aspergillus terreus* and incubated at 28°C for ten 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, Vibrio parahaemolyticus, Klebsiella oxytoca* and *Vibrio cholerae.*

Selection of the culture media

To select the suitable growth medium, the isolate *Aspergillus terreus* was grown in different culture media such as Czapek's Dox broth, Sabourod's broth, Potato dextrose broth, Malt extract broth and Nutrient broth. For biomass accumulation and bioactive metabolite production, the medium in which the isolate exhibited maximum antibiotic production expressed in terms of zone of inhibition was used as the optimized medium for further study. All the media were procured from HiMedia Laboratories, Mumbai, India.

Effect of carbon sources on biomass and bioactive metabolite production

To study the effect of different carbon sources, glucose, starch, sucrose, fructose, and maltose were used. 1% of each carbon sources were added to the basal medium individually. Each flask containing different carbon sources were inoculated with a 5 mm mycelial disc of seven days old fungal cultures and incubated for ten days. After the incubation period biomass (mycelial dry weight) and the production of bioactive metabolites were recorded Majumdar and Majumdar, (1967).

Effect of nitrogen source on biomass and bioactive metabolite production

To study the effect of different nitrogen sources, beef extract, yeast extract, peptone, ammonium chloride and sodium nitrate were used. 1% of each nitrogen sources were added to the basal medium individually and the sucrose was used as the source of carbon in all the treatments. Flasks were inoculated with 5 mm mycelial disks of seven day old fungal culture under aseptic condition and incubated for ten days. The mycelial weight and antimicrobial production were recorded at the end of the incubation period Singh *et al.,* (2009).

Effect of Temperature on biomass and bioactive metabolite production

The fungus was subjected to different temperature ranges (15 to 45°C) to study the optimum temperature required for growth and bioactive metabolite yield. Twenty five milliliters of basal medium was prepared and sterilized at 121°C at 15 *psi* for 20 minutes. Under aseptic condition 5mm diameter of the culture discs were inoculated and incubated for 10 days. After incubation the dry mycelial weight and the antimicrobial productions were recorded by Ripa *et al.,* (2009).

Effect of pH on biomass and bioactive metabolite production

The effect of pH on the growth and bioactive metabolite production of the isolate was tested in the laboratory using liquid cultures containing different pH levels (pH 5-7). Twenty milliliter of liquid medium was poured into a 150 ml conical flask under aseptic conditions. The medium was adjusted to the desired pH by adding 0.1N NaOH or 0.1N HCl Naik *et al.,* (1988). Flasks were sterilized at 121°C at 15 *psi* for 20 minutes. Each flask was inoculated with 5 mm diameter mycelial disc in sterile conditions. Inoculated flasks were incubated at 28 ± 1°C for ten days and the dry mycelial weight and bioactive metabolite productions were recorded.

Effect of NaCl concentration on biomass and bioactive metabolite production

The effect of salinity on mycelial growth and bioactive metabolite produced by the isolate *Aspergillus terreus* was carried out by incubating in various NaCl concentrations, ranging from 3-7% with 1% of carbon and nitrogen source while other parameters were kept at optimum level. The biomass as well as the bioactive metabolite production for each sodium chloride concentration were estimated and recorded.

RESULTS AND DISCUSSION

In several, metabolite biosynthesis in microbes are tightly controlled by regulatory mechanisms to avoid over production; yet, these regulatory mechanisms often sometimes process to undesirably low levels. 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 Thakur *et al*., (2009); Miao *et al*., (2006); Kumara and Rawal, (2008); Zain *et al*., (2009); Gautam *et al*., (2011); Bhattacharyya and Jha, (2011); Gogoi *et al*., (2008); Ritchie *et al*., (2009); Jain and Pundir, (2011); Sudarkodi *et al.*, (2012). Fig. 1. revealed the effect of different growth media on biomass and bioactive metabolite production. Among the tested media, maximum mycelial dry weight (74 mg/25 mL) was recorded in potato dextrose broth medium, followed by malt extract medium (55 mg/25 mL) and czapek's dox broth (51 mg/ mL) whereas sabouroud's broth and nutrient broth showed (28 mg/mL and 30 mg/mL) respectively. Similarly, maximum bioactive metabolite was produced in potato dextrose broth (23 mm against *Klebsiella oxytoca*) follwed by malt extract broth (17 mm against *Staphylococcus aureus*) and czapek's dox broth (19 mm against *Klebsiella oxytoca*). Minimum production of bioactive metabolite was observed in sabourod's broth (5 mm against *Klebsiella oxytoca*) and nutrient broth (7 mm against *Vibrio cholera*). Rabbani *et al.*, (2011) reported the potato dextrose broth medium as the best medium for the maximum growth of *Drechslera hawaiiensis*, the foliar blight pathogen of *Marsilea minuta.* Similarly the marine derived fungus *Arthrinium c.f. saccharicola* was investigated by Miao *et al*., (2006) and suggested that the culture medium had an effect on mycelial growth and metabolite profile. VUK-10 of actinomycete *Pseudonocardia* exhibited a broad spectrum of *in vitro* antimicrobial against bacteria and fungi. Production of bioactive metabolites by the strain was high in the modified yeast extract-malt extract-dextrose (ISP-2) broth as compared to other tested media Kiranmayi *et al*., (2011). Zain *et al*., (2009) reported that the growth and secondary metabolites production of *Aspergillus terreus*, *Penicillium janthinellum* and *Penicillium duclauxii* were significantly affected by the type of the growth medium and further yeast extract showed the best mycelial grown and secondary metabolite production. Whereas in the present study potato dextrose showed the best mycelial growth and bioactive compound production and Sabourod's medium showed lowest values. Similar results were reported in *Aspergillus* strain TSF 146 where maximum dry weight (71 mg/25 mL) was recorded in potato dextrose broth medium and maximum zone of inhibition (25 mm) against *Bacillus subtilis* Bhattacharyya and Jha, (2011).

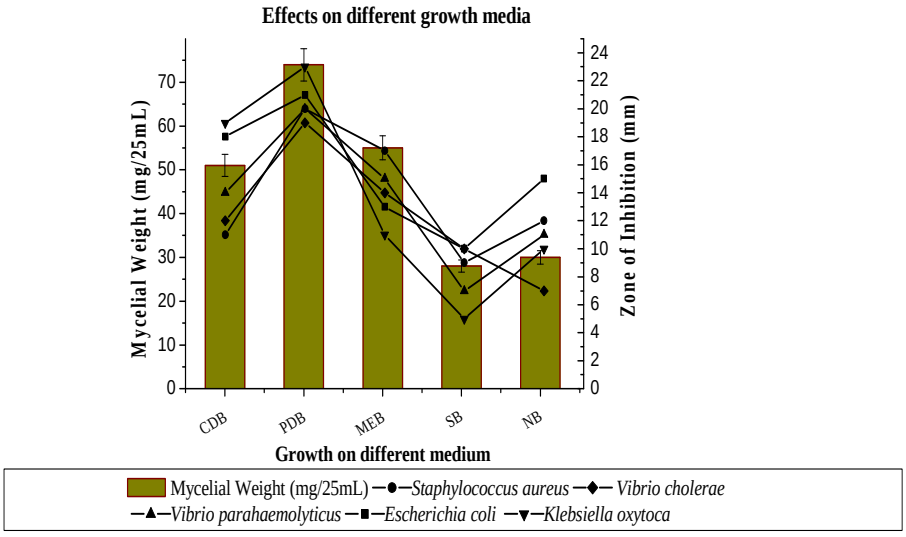

Fig. 1. Effect of different growth media on biomass and bioactive metabolite production of *Aspergillus terreus*

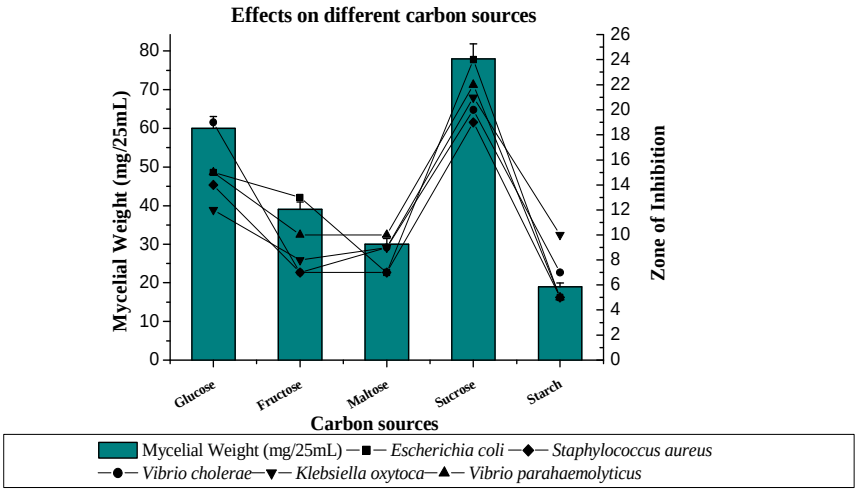

Fig. 2. Effect of different carbon sources on biomass and bioactive metabolite production of *Aspergillus terreus*

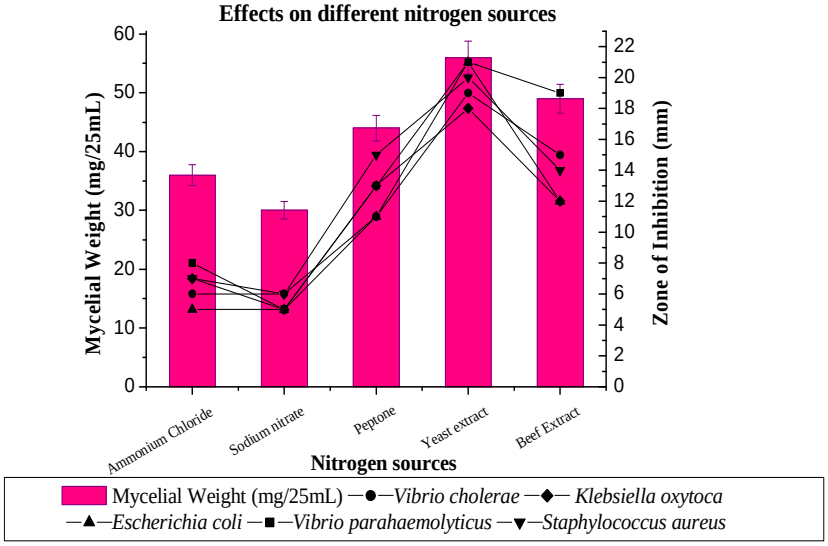

Fig. 3. Effect of different nitrogen sources on biomass and bioactive metabolite production of *Aspergillus terreus*

Fig. 2. shows the effects of different carbon sources on biomass and bioactive metabolite production by *Aspergillus terreus*. Among the various carbon sources tested, sucrose was the best carbon source for both biomass (78 mg/25mL) and bioactive metabolite production (24 mm against *Escherichia coli*). Moderate growth and bioactive metabolite production was observed in glucose supplemented media. 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). Similar results were obtained in the present study where maximum biomass and bioactive metabolite production (zone of inhibition, 24 mm) was obtained in sucrose supplemented media. Fig. 3. shows the effect of different nitrogen sources on biomass and bioactive metabolite production by *Aspergillus terreus*. Maximum biomass production (56 mg/25mL) and antibacterial activity (21 mm zone of inhibition against *Escherichia coli* and *Vibrio parahaemolyticus*) was observed in culture filtrate supplemented with yeast extract. Whereas minimum biomass production (30 mg/25mL) and antimicrobial activity (5-6 mm) in culture filtrates supplemented with sodium nitrate. 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. Yu *et al.*, (2008) reported the nitrogen source may influence the antibiotic production in *S. rimosus* MY02. Atta *et al.*, (2011) presented that the optimal antimicrobial activity was obtained with sodium nitrate in the culture medium of *Streptomyces albidoflavus* 143. Whereas in the present study medium supplemented with yeast extract showed maximum growth and bioactive metabolite production.

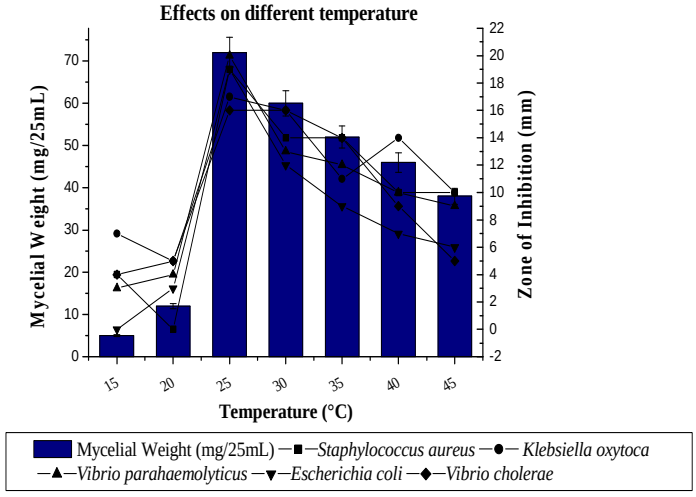

Fig. 4. Effect of different temperature on biomass and bioactive metabolite production of *Aspergillus terreus*

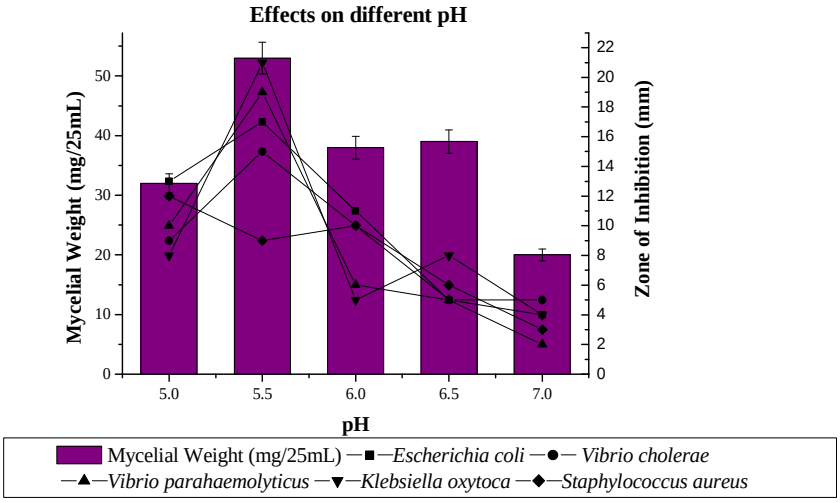

Fig. 5. Effect of different pH on biomass and bioactive metabolite production of *Aspergillus terreus*

The influence of temperature on the biomass and bioactive metabolite production of the isolate is presented in Fig. 4. Highest growth (72 mg/mL) as well as antimicrobial compound production (20 mm against *Vibrio parahaemolyticus*) was obtained at 25°C. Lowest growth and antimicrobial production was recorded at 15 and 20°C. There was a gradual decrease in biomass and antimicrobial production when the temperature was increased from 25°C to 45°C. Ritchie *et al.*, (2009) reported the incubation temperature ranging from 20°C to 25°C to be an optimum for the mycelial growth of the fungi *Rhizoctonia solani*. The increase of the incubation temperature from 25°C to 30°C enhanced the growth of the cells and production of bioactive metabolite in *Aspergillus* strain Bhattacharyya and Jha, (2011). In the present study, highest growth as well as antimicrobial compound production was obtained at 25°C. These results are in complete accordance with those reported by Jain and Pundir. (2011).

The pH of the medium determines the rate and amount of growth and other life processes Lilly and Barnett, (1951). Thongwai and Kunopakarn. (2007) pointed out the most of the microorganisms have the ability to synthesis antimicrobial compounds at pH ranging from 5.5 to 8.5. The maximum bioactive metabolite production was observed in potato dextrose broth at pH 6.0 was reported by Jain and Pudir. (2011). Gogoi *et al*., (2008) investigated the influence of pH on the growth and production of bioactive metabolite by an endophyte *Hypocrea* sp. NSF-08 isolated from *Dillenia indica Linn* in North-East, India. *C.gloeosporioides* isolates grew well at pH 5 while pH 6 was found preferred for the sporulation Kumara and Rawal. (2008). In the present study maximum growth as well as increased antimicrobial metabolites was obtained at pH 5.5 suggesting the acidophilic characteristics of the isolate. Similar results have been reported for several *Aspergillus* sp. The growth and antibacterial activity of *Aspergillus terreus* was influenced by pH of the medium (Fig. 5). Maximum mycelial growth (53 mg/25mL) and antibacterial activity (zone of inhibition with 21 mm against *Klebsiella oxytoca*) was recorded at pH 5.5. Minimum growth and antibacterial activity was observed at pH 7.0. Halotolerant marine fungal species have evolved unique metabolism to cope with the salinity change. *Arthrinium. c.f. saccharicola*, a marine derived fungus grew faster in fresh water than in seawater. High salinity condition promoted the antibacterial activity of the fungus Miao *et al.,* (2006), whereas in the present study excellent growth and bioactive metabolite production was observed at 5 g/L. The present result is in accordance with Bhattacharyya and Jha. (2011) who also reported 5 g/L as optimal for maximum mycelial growth and active metabolite production for the *Aspergillus* strain. The influence of NaCl on the biomass and bioactive compound production of *Aspergillus terreus* is presented in Fig. 6. NaCl concentration of 5 g/L was recorded as optimal for the maximum mycelial growth (51 mg/25mL) and improved active metabolite production (zone of inhibition of 23 mm against *Staphylococcus aureus*). Minimum growth and metabolite production (zone of inhibition of 7 mm against *Klebsiella oxytoca*) was recorded in the basal medium with NaCl concentration of 7 g/L.
The culture conditions have a major impact on the growth of microbes and the production of microbial product. It is evident from the above investigation that the mycelial growth and bioactive metabolite compound production by *Aspergillus terreus* isolated from the *Codium decorticatum* seaweed in the culture media is greatly altered. The maximum growth and bioactive compound could be achieved in potato dextrose medium supplemented with sucrose as a carbon source and yeast extract as a nitrogen source *in vitro.* Further process parameters like incubation temperature at 25°C, pH 5.5 NaCl at 5 g/L are found to be optimum for the maximal mycelial growth and bioactive metabolite production.

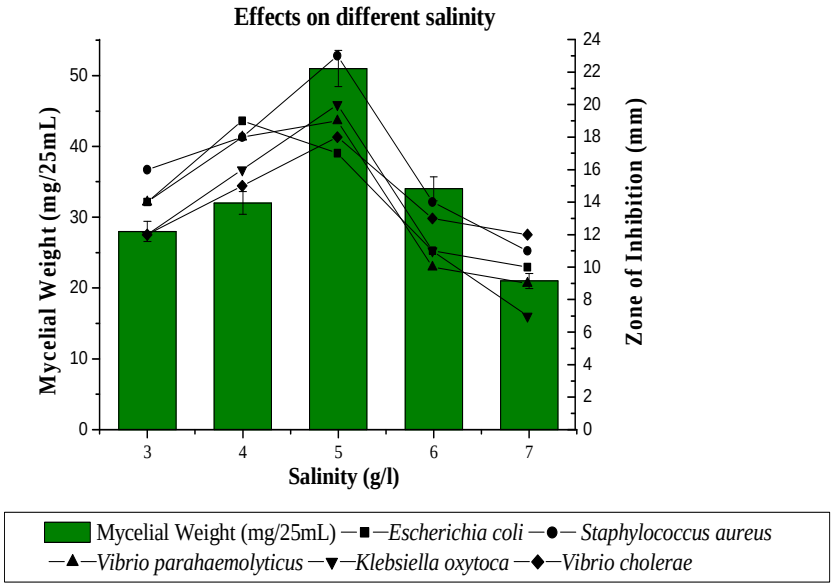

Fig. 6. Effect of different salinity on biomass and bioactive metabolite production of *Aspergillus terreus*

CONCLUSION

In the present study, concluded that the optimum conditions required for the production of bioactive metabolite by seaweed endophytic fungi *Aspergillus terreus* KC 582297 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 terreus* KC 582297.

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