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Effect of different carbon sources on production of antimicrobial metabolite from *Aspergillus ibericus*

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The present study highlights the effect of different carbon sources on the production of antimicrobial metabolite of rhizosphere soil fungus*Aspergillus ibericus* from *Ficus religiosa*.The various carbon sources used in the fermentation medium were glucose, dextrose, fructose, sucrose and lactose. Antimicrobial metabolite obtained from *A. ibericus* was found to be effective against test microbes when dextrose was used as a best carbon source. For evaluating the most suitable concentration of selected carbon source, dextrose for antimicrobial metabolite production, potato broth was supplemented with different concentration (2, 3, 4, 5 and 6%) of dextrose. The antimicrobial metabolite production was found to be effective at 4% dextrose concentration as measured by growth inhibition of various test microbes used in the experiments. *Aspergillus ibericus* antimicroboal metabolite/s may be used as an alternative in place of commercially available antibiotics.

Keywords : Antimicrobial resistance, *Aspergillus ibericus*, opportunistic pathogens, rhizosphere soil

INTRODUCTION

Antibiotics are the most important pillar of current medications.The antimicrobial compounds have been found to cure various kind of bacterial and fungal infections, but the discovery of these agents has been tempered by appearance of resistant microbial pathogens. Emergence of drug resistance in them has emphasized the need of research for new compounds. Antibiotics nowadays are taken for granted to treat bacterial infections. The antibiotics are successfully used for prophylactic or therapeutic purposes regularly in clinical, veterinary and agricultural purposes. However, overconsumption of antibiotics caused an enormous selective pressure on the bacteria to gain resistance or die (Ventola, 2015). The increasing frequency of multi-resistant pathogenic bacteria has compromised the clinical treatment of an emerging infectious diseases. There is an urgent demand for new antimicrobial compounds active against current resistant pathogens and emerging pathogens.Natural drug discovery involves the search for natural sources such as soil, bacteria, mold and plants for new chemical entities (NCE). Natural products provide a vast source of chemically diverse biologically active leads for therapeutic agents.Most of the antibiotics used all over the world are derived from natural compounds (Prestinaci *et al.* 2015).

Antibiotic formation usually occurs during the late growth phase of the producing microorganism. The temporal nature of their formation is certainly genetic, but expression can be influenced greatly by environmental manipulations. Therefore, synthesis of antibiotics is often brought on by exhaustion of a nutrient, addition of an inducer and/or by a decrease in growth rate (Sanchez and Demain, 2002). Formation of antibiotics is also regulated by nutrients (such as nitrogen, phosphorous and carbon source), metals, growth rate, feedback control and enzyme inactivation. Among these nutrients, the effect of carbon sources on antibiotic production has been the subject of continuous study for both industry and research groups, not only from fermentation but

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also from biochemical and molecular biological stand points (Zhu, 2017). The present study highlights the effect of carbon sources and their different concentrationson antimicrobial metabolite production by rhizospheric soil fungus *Aspergillus ibericus* obtained from *Ficus religiosa* against opportunistic pathogens.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from rhizosphere of medicinal plant Peepal (*Ficus religiosa*) from Botanical garden, Kurukshetra University, Kurukshetra (Haryana)by removing 1-1.5 inch of top soil with sterilized spatula.

Isolation of fungus, Aspergillus ibericus from soil

The serial dilution agar plate method was used for isolation of *Aspergillus ibericus* from soil sample. Potato dextrose agar (PDA) was used for fungalisolation. One gram of soil (finely pulverized and air dried) was suspended in 9ml sterilized distilled water blank no. 1 and shaken vigorously to obtain uniform suspension. 1ml of suspension was transferred, while in motion, from stock suspension (No. 1) to sterile water blank no. 2 with sterile pipette under aseptic conditions to make 1:100 (10^{-2}) dilution. For fungi, 10^{-2} to 10^{-5} dilutions were used. Approximately 20-25ml cooled (45°C) molten potato dextrose agar was added to each Petri plate. After solidification of agar media, 100µl aliquots of suspension of dilutions were added to labeled and sterilized petriplates and spread with spreader. Inoculated plates were incubated at 30°C for 4-5 days for fungi (Aneja, 2003).

Purification and maintenance of isolates

Fungal colonies appearing on their respective medium were transferred to potato dextrose agar plates (one colony on each plate) at 30°C for 4-5 days. The colonies were then transferred on potato dextrose agar slants and incubated at 30°C for 4-5 days for fungal isolate and were maintained at 4°C in a refrigerator for further studies (Aneja, 2003).

Effect of carbon sources on antimicrobial metabolite production

For evaluating the most suitable carbon source for antimicrobial metabolite production, potato broth was supplemented with various carbon sources such as glucose, dextrose, fructose, sucrose and lactose. Each flask with 100ml of potato broth supplemented with different carbon sources was autoclaved at 121°C for 15 minutes. For each source, three replicates were used. Three disks cut from four days old colony of selected fungus were added as inoculum in each flask. The inoculated flasks were incubated at 30°C for 12-14 days under stationary condition. The extracts after filtration with filter paper were assayed for antimicrobial activity against test microbes by using agar well diffusion method (Arora *et al.* 2014; Olfat and Sadik, 2015).

Effect of carbon source concentration on antimicrobial metabolite production

For evaluating the most suitable concentration of selected carbon source for antimicrobial metabolite production, potato broth was supplemented with different concentration (2, 3, 4, 5 and 6%) of selected carbon source. Each flask with 100ml of potato broth supplemented with different concentration of selected carbon source was autoclaved at 121°C for 15 minutes. For each source, three replicates were used. Three disks cut from four days old colony of selected fungus were added as inoculum in each flask. The inoculated flasks were incubated at 30°C for 12-14 days under stationary condition. The filtrate after filtration with filter paper was evaluated for antimicrobial activity against test microbes by using agar well diffusion method (Arora *et al.* 2014; Olfat and Sadik, 2015; Xu *et al.* 2017).

Statistical analysis

The data obtained from experiments of optimization of carbon and nitrogen sources and their concentration were subjected to analysis of

variance (One Way ANOVA) to evaluate the significance of each parameter by estimating p-value and f-value. The level of significance was considered as $p<0.05$.

RESULTS AND DISCUSSION

In the present study, the fungus *Aspergillus ibericus* was isolated from rhizosphere soil of medicinal plant *Ficus religiosa* and was studied for effect of carbon sources in fermentation medium on antimicrobial potential against test microbes *B. subtilis, S. aureus, S. mutans, S. pyogenes, P. aeruginosa, E. coli* (bacteria) *C. albicans* and *C. tropicalis* (yeasts) by agar well diffusion method, which could cause opportunistic infections in host.

Ramos and Said (2011) reported that by changing the constituents of fermentation medium, antibacterial activity of the fungus could be improved. Variation in the type of carbon sources play important roleas microbial and fermented products are largely composed of these elements. Therefore, optimization and concentration of proper nutrients are necessary conditions to achieve maximum production of antimicrobial metabolites by microbial strain.Alterations of some of the nutrients in the culture media may cause some positive effects on the growth of the strain.

In the present study, different monosaccharide carbon sources such as glucose, dextrose, fructose and disaccharides such as lactose and sucrose were added in basal media to optimize the carbon source for maximum antibiotic production by isolated strain *A. ibericus*. Maximum activity was shown by strain in basal medium amended with dextrose with zone of growth inhibition 24mm against *B. subtilis*, 24mm against *S. aureus*, 25mm against *S. mutans*, 27mm against *S. pyogenes*, 27mm against *P. aeruginosa*, 24mm against *E. coli*, 27mm against *C. albicans* and 22mm against *C. tropicalis*. Antimicrobial activity was decreased in medium amended with glucose (17mm to 24mm), fructose (21mm to 24mm), lactose (17mm to 22mm) and sucrose (0mm to 20m). One-way ANOVA analysis at 5% significance level shows calculated F value (7.44) greater than F critical

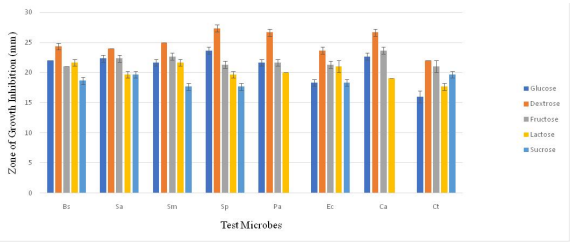

Fig. 1 : Optimization of carbon source and antimicrobial activity Bs: *Bacillus subtilis*; Sa: *Staphylococcus aureus*; Pa: *Pseudomonas aeruginosa*; Ec: *Escherichia coli*; Sm: *Streptococcus mutans*; Sp: *Streptococcus pyogenes*; Ca: *Candida albicans*; Ct: *Candida tropicalis*. When statistically analyzed at significance level 0.05 by One Way ANOVA, proved to be significantly different.

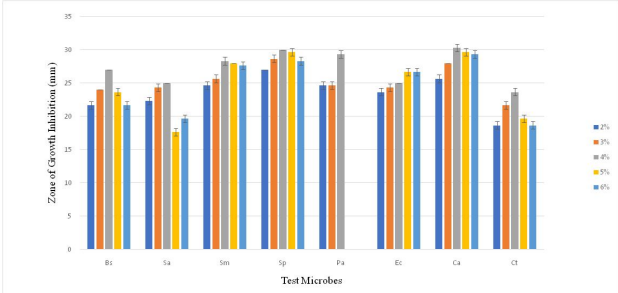

Fig. 2. Optimization of dextrose concentration and antimicrobial activity Bs: *Bacillus subtilis*; Sa: *Staphylococcus aureus*; Pa: *Pseudomonas aeruginosa*; Ec: *Escherichia coli*; Sm: *Streptococcus mutans*; Sp: *Streptococcus pyogenes*; Ca: *Candida albicans*; Ct: *Candida tropicalis*. When statistically analyzed at significance level 0.05 by One Way ANOVA, proved to be non-significant.

Fig. 3.Optimization of dextrose concentration and antimicrobial activity against test microbes A), B) *S. mutans* and C), D) *S. pyogenes*; 1: 2%, 2: 3%, 3: 4%, 4: 5% and 5: 6%

value (2.64) and P value (0.00019) less than 0.05, which indicates that null hypothesis (there is no significant difference between the values) is rejected and there is significant difference between values. Table1 and Fig.1 show antimicrobial activity of fungus *A. ibericus* incubated in media supplemented with different carbon sources. The results of this study correlate with the results of Merlin *et al*. (2013) who optimized the growth and bioactive metabolite production of fungus *Fusarium solani* and found dextrose as best and stable carbon source for higher biomass and bioactive metabolite production. Addition of glucose resulted highest growth of the fungus, but significantly less bioactive metabolite production.

Table. 1: Optimization of carbon sources and antimicrobial activity

Carbon source	Zone of growth inhibition (mm) Test microorganisms							
	Bacteria						Yeast	
	Gram-positive			Gram-negative				
	Bs	Sa	Sm	Sp	Pa	Ec	Ca	Ct
Glucose	22.00±0.00	22.33±0.57	21.66±0.57	23.66±0.57	21.66±0.57	18.33±0.57	22.66±0.57	16.00±1.00
Dextrose	24.33±0.57	24.00±0.00	25.00±0.00	27.33±0.57	26.66±0.57	23.66±0.57	26.66±0.57	22.00±0.00
Fructose	21.00±0.00	22.33±0.57	22.66±0.57	21.33±0.57	21.66±0.57	21.33±0.57	23.66±0.57	21.00±1.00
Lactose	21.66±0.57	19.66±0.57	21.66±0.57	19.66±0.57	20.00±0.00	21.00±1.00	19.00±0.00	17.66±0.57
Sucrose	18.66±0.57	19.66±0.57	17.66±0.57	17.66±0.57	0.00±0.00	18.33±0.57	0.00±0.00	19.66±0.57

Values are mean inhibition zone ± Standard deviation of three replicates
Bs: *Bacillus subtilis*; Sa*: Staphylococcus aureus*; Sm: *Streptococcusmutans*; Sp: *Streptococcus pyogenes*; Pa: *Pseudomonas aeruginosa*; Ec: *Escherichia coli*; Ca: *Candida albicans*; Ct: *Candida tropicalis*

Table 2 : Optimization of dextrose concentration and antimicrobial activity

Dextrose conc.	Zone of growth inhibition (mm) Test microorganisms							
	Bacteria						Yeast	
	Gram-positive			Gram-negative				
	Bs	Sa	Sm	Sp	Pa	Ec	Ca	Ct
2%	21.66±0.57	22.33±0.57	24.66±0.57	27.00±0.00	24.66±0.57	23.66±0.57	25.66±0.57	18.66±0.57
3%	24.00±0.00	24.33±0.57	25.66±0.57	28.66±0.57	24.66±0.57	24.33±0.57	28.00±0.00	21.66±0.57
4%	27.00±0.00	25.00±0.00	28.33±0.57	30.00±0.00	29.33±0.57	25.00±0.00	30.33±0.57	23.66±0.57
5%	23.66±0.57	17.66±0.57	28.00±0.00	29.66±0.57	0.00±0.00	26.66±0.57	29.66±0.57	19.66±0.57
6%	21.66±0.57	19.66±0.57	27.66±0.57	28.33±0.57	0.00±0.00	26.66±0.57	29.33±0.57	18.66±0.57

Values are mean inhibition zone ± Standard deviation of three replicates
Bs: *Bacillus subtilis*; Sa*: Staphylococcus aureus*; Sm: *Streptococcusmutans*; Sp: *Streptococcus pyogenes*; Pa: *Pseudomonas aeruginosa*; Ec: *Escherichia coli*; Ca: *Candida albicans*; Ct: *Candida tropicalis*

The simple carbohydrates like glucose, dextrose through metabolic pathway affects the production of intermediates leading to primary as well as secondary metabolites in addition to water and energy. The production of antibiotic is promoted after readily utilizable sugars are used as a carbon sources. The highest antibacterial activity of *Streptomyces sannanensis* strain RJT-1 was obtained when glucose at 1% (w/v) was used as a carbon source followed by xylose and arabinose (Sanchez and Demain, 2008; Singh and Rai, 2012).

After the selection of best carbon source dextrose, its various concentration (2%, 3%, 4%, 5%, 6%) were added in medium to optimize the concentration of dextrose for maximum antimicrobial metabolite production. Maximum activity was observed at 4% dextrose concentration with zone of growth inhibition 27mm against *B. subtilis*, 25mm against *S. aureus*, 28mm against *S. mutans*, 30mm against *S. pyogenes*, 29mm against *P. aeruginosa*, 25mm against *E. coli*, 30mm against *C. albicans* and 23mm against *C. tropicalis* by antagonistic strain. The antimicrobial activity was comparatively less at concentration of dextrose at 2% (19mm to 27mm), 3% (22mm to 29mm), 5% (0mm to 30mm) and 6% (0mm to 30mm). The antimicrobial activity increased from 2% to 4% dextrose then decreased from 4% to 6% dextrose concentration. One-way ANOVA analysis at 5% significance level shows calculated F value (1.12) less than F critical value (2.64) and P value (0.362359) greater than 0.05, which indicates that null hypothesis (there is no significant difference between the values) is accepted and there is no significant difference between values.Table 2 and Fig.2 show antimicrobial activity of fungus *A. ibericus* incubated in media supplemented with different concentration of dextrose. Antimicrobial activity of fungus *A. ibericus* incubated in media supplemented with different concentration of dextrose against test microbes *S. mutans* and *S. pyogenes* are shown in Fig. 3. The antibiotic production decreased with increase in its concentration while the cell biomass increased. This might be due to its suppressive effect on production of secondary metabolites, also reported by Arora *et al*. (2014). Maximum antimicrobial activity was observed at 1% dextrose which decreased at 4% and showed no activity at all at 6% against any bacteria. Similarly, maximum biomass was obtained at the highest concentration of starch i.e. 10% while the maximum antimicrobial activity was observed at 1% starch which declined with further increase in the concentration. The cell metabolism in actinomycetes under conditions of excess of nutrients is directed towards the generation of biomass rather than the secondary metabolites production. But the condition where depletion of key nutrients occurs, it shifts the cell cycle to the stationary phase and signals the transition from primary to secondary metabolism in which antimicrobial metabolites are produced.Higher concentration of glucose showed negativeeffect on production of active metabolites in many fermentation processes.Reddy *et al*. [also reported that lower concentrations of peptone (1%) mainly favored higher antimicrobial metabolite yield by *Streptomyces rochei*. Geetanjali *et al*.(2020) reported the isolation, purification and characterization of Antimicrobial Metabolite from *Aspergillus ibericus*. Tan *et al*. (2022) worked on effects of different carbon sources on the oxidative stress tolerance of *Aspergillus niger* HY2 isolated from spoiled paddies. Singab et al. 2023 also reported antimicrobial activities of metabolites isolated from endophytic Aspergillus flavus of Sarcop hytonehrenbergis supported by in-silico study and NMR spectroscopy. Hamed et al (2014) worked on induction of antimicrobial, antioxidant metabolites production by co-cultivation of two red-sea-sponge- associated *Aspergillus* sp. CO2 and *Bacillus* sp. COB Z 21.

CONCLUSION

It may be concluded that fungus *Aspergillus ibericus* isolated from rhizosphere soil of medicinal plant *Ficus religiosa* is a promising source of antimicrobial metabolite and exhibits maximum antimicrobial activity in the presence of dextrose at 4% concentration. It may also be suggested that further research is needed to determine the cytotoxicity and in vivo efficacy against opportunistic pathogens before it is used for commercialization purpose.

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DECLARATIONS

Conflict of interest. Authors declare no conflict of interest.

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