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Research Article

Optimization of Fermentation and Production of Antimicrobial Agents as Secondary Metabolites from an Indigenous *Aspergillus niger*

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FA conducted the research. MZ supervised the research work while ZA co-supervised the work. RTM was the research collaborator.

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Antimicrobial agents, Secondary metabolites, Fermentation, Tautomycetin, *Aspergillus niger*

Abstract | Fungal strains are being exploited extensively for their potential in synthesizing antimicrobial agents. In the present research work, four fungal strains *Rhizopus stonolifer*, *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus terreus* were tested for the production of antimicrobial agents. The produced antimicrobial metabolites included Aflatrem, Monacolin K, Fusarubin and Tautomycetin. Different fermentation parameters were optimized to produce antimicrobial agents using Response Surface Methodology (RSM) and best results were obtained at 45°C temperature, 5.5 pH, 5 mL inoculum concentration, 1.5 mL banana peel as substrate and 20 days incubation time. The antimicrobial activity of secondary metabolites was tested against *Streptococcus aglactaie, Streptococcus pyogene, Escherichia coli* and *Proteus mirabilis*. The fungus *Aspergillus niger* was found best among all tested strains as it produced maximum amount of antimicrobial agent Tautomycetin. The antimicrobial activities were determined through disk diffusion assays and Tautomycetin was observed as best antimicrobial agent having inhibitory effect on serine/threonine phosphatases leading to hindrance in bacterial growth. In addition, phylogenetic tree was also constructed using 18s rRNA gene of *A. niger* and gene clusters of secondary metabolites were determined through bioinformatics tools.

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Introduction

Term antibiotic is defined as 'against life' or can be defined as any metabolite which is secondary in nature being isolated from any microbe which can exhibit any antimicrobial activity; which can be (antifungal, antibacterial, antiprotozoal, antiviral or antitumor). Up till now, there are about more than 16500 antibiotics obtained from different sources, 29% of these antibiotics are obtained from different strains of fungi. Among molds '*Aspergillus*' is found to have high aptitude of producing antibiotics (El-Elimat *et al*., 2012).

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The infectious diseases have been a great hazard for human health since millions of years (Kristiansson *et al*., 2011). They have been a lifelong threat in the form of pandemics, endemics and epidemics. A recent element in this regard is emergence of resistance to currently available conventional antibiotics including antibacterial, antifungal, antiviral drugs for the prophylaxis and for the treatment of infectious diseases. This concern continues to spur the development and search of novel and putative antimicrobial agents to modify and supplement the existing therapeutic approaches. Different fungi have been employed for production of antibiotics. Species like *Penicillium chrysogenum, Penicillium griseofulvin, Aspergillus terreus, Aspergillus fumigatus, Aspergillus niger, Trichoderma polysporum* and *Cylindrocarpon lucidum* have been exploited by many researchers for antibiotic potential (Kristiansson

et al., 2011). The beneficial property of fungi was exploited by Sir Alexander Fleming in 1928 who found that *Penicillium notatum* can produce a substance which is far more capable than any other chemical agent available to kill Gram positive bacteria. From that time until now molds are widely exploited for producing antibiotics which are being used in various current chemotherapy. For millennia, humans have extracted and used substances of natural origin as penicillin, morphine, and caffeine to improve their quality of life. Many important medicines are natural products or derived from natural products (Newman and Cragg, 2012). More than one third (39.1 %) of all FDA-approved drugs are of natural origin and 48.6 % of all cancer drugs registered from the 1940s until today are either natural products or derivatives thereof. With the development of modern analytical chemistry, microbiological cultivation methods, assays, and genetics during the last 50 years, the ability to isolate natural products from plants and microorganisms has never been better (Cragg and Newman, 2013). Combined with molecular biology and computer modeling, this field of research will continue to be a significant source of useful bioactive compounds (El-Elimat *et al.*, 2012). From that time, antibiotics produced by molds are widely used in chemotherapy. Most commonly used are cephalosporin, penicillin and fusidic acid which constitute both antifungal and antibacterial activity. Different strains or species of *penicillium* are now been identified for producing active metabolites which have antifungal, antitumor and antibacterial activity (Midgley *et al.*, 2007). The 18S rRNA are known extensively for the molecular taxonomic studies of fungi. Different growth condition like pH, temperature, incubation time and nutrients greatly influence the cultivation and hence the fungal strain capable of producing antimicrobial bioactive metabolite substance. For that reason, different environmental factors need to be optimized properly for high yield of antimicrobial agent(s) (Iwai and Omura, 1982). Therefore, it is important to identify compounds produced and find out the pathways leading to their formation in microbes. In this connection, production of several antimicrobial agents from fungi has been reported in the present research work.

Materials and Methods

Sampling and cultivation

Different fungal strains were used to produce antimicrobial agent and were isolated from different dietary sources including rotten fruits, vegetables and decayed bread from different sites in Pakistan. The filamentous fungi were isolated by using sterilized steel loop to pick and spread samples over potato-dextrose agar plates in Industrial Biochemistry Lab, Dept. of Biochemistry and Biotechnology, University of Gujrat, Gujrat, Punjab, Pakistan. After streaking plates were incubated at 37°C for 3-5 days until the colonies become visible. These strains were re-cultured on PDA medium since isolation of a single and pure colony of each fungal strain. The fungal strains were identified morphologically followed by molecular identification through 18s rRNA sequencing (Chang *et al.*, 1992).

Screening for antagonistic activity

Each of four strains was cultivated in potato-dextrose broth and incubated at 37°C temperature, 140 rpm for 7 to 20 days to find out optimum incubation time for the secretion of secondary metabolites (Al-Jawad *et al.*, 2015).

Primary screening

Primary or the preliminary screening for antimicrobial activity was conducted through disk diffusion assay. *Streptococuus aglactaie* was taken as control bacterial pathogen. A test bacterium was spread by cotton swab on solidified LB medium. After this, bore of 7mm was made at the center of media with the help of cork-borer. Secondary metabolite was extracted from four fungal strains and poured into LB medium. It was kept at 27°C for 3 hours which allowed secondary metabolite to flow into the media. The plates were placed in incubator at 37°C for 24 hours after which their zone of inhibition was measured (Omran, 2015). On the base of inhibition zone, *Aspergillus niger* was observed with maximum ability to produce antibiotic among all strains (Khamna *et al.*, 2009).

Substrate screening

Five different substrates (apple peel, molasses, banana peel, lemon peel and orange peel) were used in order to find best one regarding maximum production of secondary metabolites. Substrates were dried under sunlight for 8 to 10 days, grinded and preserved in plastic bags. Five grams of each substrate was taken in a flask with the equal amount of autoclaved distilled water with maintained pH of 7 using glycine and phosphate buffers. Each substrate was inoculated with 3ml of culture of *A. niger* and placed in incubator at 37°C for 7, 14 and 20 days. The flasks were placed in orbital shaker (120 rpm) for 2 hours at room temperature, after addition of 50 mL distilled water. The solution containing secondary metabolite was filtered with the help of filtration Assembly (Millipore filter 0.22µm). The filtrate was evaporated using rota-evaporator at 75°C. After evaporation secondary metabolite residues were obtained and preserved in 70% ethanol. Different concentrations of each sample content were subjected to test tubes containing 3ml of test bacterial colonies. One test tube containing bacterial colonies was considered as control. It was incubated at 37°C for 6 hours, after incubation each sample was analyzed by UV-Visible spectrophotometer at 600 nm to check reduction in the number of colonies of bacterial specie. For further confirmation, sample yielding maximum absorption during spectral analysis was subjected to disk diffusion assay. Bacterial strains were spread on bored-LB plates. Samples

were poured onto bore and allowed to penetrate medium at 27˚C followed by incubation of petri plates at 37˚C for 24 hours. The zone of inhibition was checked through measurement and spectral readings (Khamna *et al*., 2009).

Optimization of fermentation parameters

Different parameters were optimized for the maximal production of antimicrobial agents through fungal cultures. For temperature optimization, culture medium was incubated at various temperatures ranging from 25 to 50° C and antibiotic activity was measured via measuring the zone of inhibition and spectrophotometry. The combined effect of pH, substrate and inoculum concentration was checked by applying these parameters in varying sets of combinations onto the production media which was again inoculated via selected fungal strain. The results were observed after completion of 20 days at 50° C. The secondary screening was performed following the above-mentioned optimized conditions of various parameters for production of antimicrobial agents from selected fungal strain and activity of antimicrobial agent was tested onto four different bacterial pathogenic strains through measurement of diameter of inhibition zone (Jain and Pundir, 2011).

Molecular identification

Genomic DNA extraction

The fungal isolate was cultured on PDA medium for 5 days at 37°C. Fungal DNA isolation kit (Mo Bio Laboratories, Inc.) was used for extraction of DNA which was purified using beads powder that are highly effective in mold cell lysis (Guo *et al*., 2007). Fast Prep FP120 device was used for 30 seconds at speed of 5 to disrupt cell membrane contents in the presence of PCR and SDS inhibitors removal. Extraction procedure was used in accordance to the guideline of manufacturer. The DNA concentration and percentage purity were measured using nanodrop ND-1000 UV-spectrophotometer. Extracted DNA of mold was preserved at -20° C till further use (Nguyen *et al*. 2017).

Amplification and sequencing of 18s rRNA gene

18s rRNA gene was amplified using universal primers. The purified PCR product was sequenced using BigDye® Terminator v3.1 sequencing reaction Kit. The reaction mixture was maintained up to 10µl of final reaction. 18s universal primers were used for sequencing of the selected isolated mold. Reaction tubes were transferred to BioRad C1000 thermal cycler. The purified DNA was transferred to ABI prism 3130XL genetic analyzer for recording the sequence. Sequencing results were analyzed for checking the presence of chimeras via Bellerophon program (Huber *et al*., 2004).

Phylogenetic analysis

The sequence was analyzed for its similarity with other sequences available in National Center for Biotechnology Information (NCBI) database via aligning the test sequence with other suitable sequences and carrying out their multiple sequence alignment with the help of Nucleotide Basic Local Alignment Search Tool (nBLAST). This tool was selected for carrying out the analysis. Based on E-score of blast results, 10 highly similar sequences were selected to determine their phylogenetic relationship. A phylogenetic tree was constructed following the method of Saitou and Nei (1987) using MEGA 7 software (Tamura *et al*., 2011) through neighbor-joining method with 2000 selected bootstrap.

Predictions of KS and C domains

Natural Product Domain Seeker (NaPDos) was used to carry out rapid detection and analysis of secondary metabolite genes. This tool is specifically designed for detection of KS and C-domain from within the sequence. Test sequence secondary metabolic domains were identified by comparing sequences with sequences of reference genes of well-defined chemical pathways. Sequence of the candidate gene was firstly extracted, trimmed and translated which was subjected to domain specific phylogenetic clustering. Possible gene products were predicted under study by investigating similarity of genes to elucidate relational similarity of their biosynthetic pathways to produce similar compound (Ziemert *et al*., 2012). The software predicted 6 KS domains and 5 C-domain within sequence. For further confirmation anti SMASH tool was used that also analyzed the presence of secondary metabolite genes within the sequence. This tool used different approaches to search for gene clusters by comparing it with other sequence capable of producing secondary metabolite. The gene clusters present in sequences were involved in the formation of secondary metabolite. By comparing within the record, four different biosynthetic pathways leading to the formation of secondary metabolite were identified and five types of secondary metabolite were detected (Weber *et al*., 2015).

Docking analysis

Auto dock vina was employed for carrying out docking analysis of these compounds with the test bacterial sequence genome to find its possible interacting positions and combination to elucidate the combinatorial effect of these compounds on bacterial survival (Trott and Olson, 2010).

Results and Discussion

The main objective of this research work was to produce antimicrobial secondary metabolites through cheap and renewable agricultural wastes using Solid State Fermentation (SSF). Molasses, banana peel, apple peel, orange peel and lemon peel were used as carbon sources. *Aspergillus niger* was used to produce antimicrobial agents as fungal secondary metabolites. The production

of antimicrobial agents was enhanced by optimization of different parameters under Response Surface Methodology (RSM) technique.

Morphological identification

Fungal strains are found extensively in our environment, among which *A. niger* has been most prevalent (Netzker *et al.*, 2018; Höller *et al.*, 2000). During preliminary identification of strains, obtained from various sources like rotten fruits and bread molds, the morphology was compared to other strains already reported. Four different strains were identified which were analyzed for their ability to produce antibiotic as secondary metabolites. Morphological analysis showed F1 to be *Rhizopus stonolifer*, F2 to be *Aspergillus niger*, F3 to be *Aspergillus fumigatus* and F4 as *Aspergillus terreus* (Fig. 1). The reported method for morphological identification of fungal strains isolated from different habitats has been used by many scientists (Pereira *et al.*, 2013).

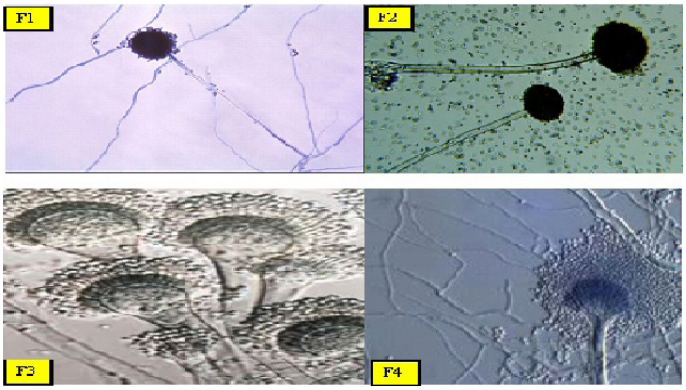

Figure 1: Four different indigenous fungal species isolated and observed under light microscope (Accuscope 3012 LED Binocular Microscope, Accuscope INC.), F1; *Rhizopus stonolifer*, F2: *Aspergillus niger*, F3; *Aspergillus fumigatus* and F4; *Aspergillus terreus*.

Primary Screening of fungi for maximum production of antimicrobial agents

(F1) *Rhizopus stonolifer*, (F2) *Aspergillus niger*, (F3) *Aspergillus fumigatus* and (F4) *Aspergillus terreus* were subjected to grow on PDA inoculum at 37°C for 20 days. PDA media has been declared as best by many researchers for primary screening of antibiotic producing mold (Pereira *et al.*, 2013). The secondary metabolites were successfully preserved after evaporation of fungal contents followed by mixing in 70 % ethanol. The respective zones of inhibition were measured against *Streptococcus aglactaie* (Control). *Rhizopus stonolifer* (F1), *Aspergillus niger* (F2), *Aspergillus fumigatus* (F3) and *Aspergillus terreus* (F4) produced zones of inhibition of 7, 8, 7.5 and 7 mm, respectively. As *Aspergillus niger* produced largest zone of inhibition as compared to others, therefore this strain was highly capable of producing secondary metabolites having maximum inhibitory effect onto the growth of bacterial strains. These results suggest that this strain could also be used for other bacterial strains, due to its potential. Our finding is in accordance with other researchers which conducted various trials to screen fungal strains to produce antimicrobial agents and observed that test pathogen, producing strain and solvent used for extraction have significant effect on production of secondary metabolites as antimicrobial agents. Our selected strains are commonly available and have evidence for producing antimicrobial agents (Zeilinger *et al.*, 2016). Like our finding, species of *Aspergillus* have been reported for producing many bioactive compounds including mycotoxins (Yu, 2012). In addition, antimicrobial agents like Lovastatin, Amodin A, Amodin B, Tensyuic acids A to F, Stemphones B, C, D, G, Tensidols, Flavasperone and Sterigmatocystin have also been reported previously as bioactive compounds produced from *Aspergillus* species (Zeilinger *et al.*, 2016).

Optimization of substrate, dosage and incubation time

Various parameters were optimized to produce maximum number of secondary metabolites per 10µL of crude extract. The growth pattern was observed for each substrate for 7, 14 and 20 days. Optical density of each sample was checked and compared to control. Using PDA medium, and incubated for 14 days, *A. niger* showed maximum optical density of 0.516 followed by 0.53 value obtained using molasses as substrate, 7 days incubation period and 20µL concentration of fungal crude extract. When orange peel was used as substrate, incubated for 7 days with 10µL concentration of fungal crude extract, the value of optimal density was 0.528. The lemon peel with 14 days of incubation and 100 µL concentration of fungal extract exhibited 0.479 value of optimal density. The maximum production of secondary metabolites by *A. niger* was observed in the presence of apple peel as substrate and 14 days incubation period with 30 µL crude extract, as it exhibited 0.60 value of optimal density. The banana peel with 100 µL concentration of crude extract showed decrease in bacterial colonies due to production of secondary metabolites as the OD value was decreased to 0.453. Our results indicate that banana peel was best substrate to produce secondary metabolites from *A. niger*. The specified length of each microbial lag phase and its metabolism varies with the change in chemical composition of lignocellulosic substrate which influences amount and time required for production of secondary metabolites (Koser *et al.*, 2014). In addition, other factors like organism, nature of medium, nutrients concentration and physiological conditions have also been reported to have strong influence on production of secondary metabolites from fungal cultures (Joshi *et al.*, 2006). The results were further confirmed through Disk-diffusion assay.

Disk-diffusion assay

There are different bioassays available for detecting antimicrobial activity among which disk diffusion assay

has been most common. This assay has been employed by many researchers to check the sensitivity of pathogens (Humphries *et al*., 2018). Fungal crude secondary metabolites were subjected to Disk-diffusion assay. Antibiotic could spread at normal room temperature for 3 hrs, petri plates were placed in incubator at 37°C for 18 hours and respective zone of inhibition was measured. The values of measurement of zone of inhibitions were in accordance with the values obtained through spectral analysis. Molasses and lemon peel extract showed no zone of inhibition while Apple peel and Orange peel extracts showed 12mm and 13mm zone of inhibition, respectively. The maximum zone of inhibition (15 mm) was observed when banana peel was used as substrate.

Optimization of temperature

After optimization of substrate, dosage and incubation time, the maximum production of antibiotics as fungal secondary metabolites was subjected to variable range of temperature. The increased production of antibiotic per µL of crude fungal extract requires minimum dosage against bacterial pathogen. The production was observed at different ranges of temperature from 25°C to 50°C using optimized conditions of other parameters as discussed above. The results exhibited that maximum production of antibiotics was observed at 45°C. The results of antimicrobial activity of fungal extracts were again verified through disk diffusion assay. *Steptococcus aglactaie* as test microorganism was streaked onto the media after which crude fungal extract was poured. The petri plates were placed in incubator at 37°C for 18 hours and zone of inhibition of each extract treated with different temperature was noted. The zone of inhibition at 25°C, 30°C, 35°C 40°C, 45°C and 50°C was 21mm, 24mm, 25mm 26mm, 28mm and 27.5mm respectively. The maximum zone of inhibition (28 mm) was produced at 45°C which confirms the above-mentioned findings regarding production of antimicrobial agents through extract of *A. niger*. The effect of temperature has been considered most obvious physiological condition influencing the synthesis of respective metabolites from a microorganism (Rodrigues *et al*., 2018).

Optimization of substrate, pH and inoculum concentration

Response Surface Methodology (RSM) was employed to optimize the parameters of substrate, pH and inoculum concentration and pH as this design investigates relation among explanatory and response variables allowing multiple parameters to be implied in combination. All the parameters were optimized and evaluated for production of secondary metabolites. The parameters of pH, substrate and inoculum concentration were variably implied in 20 different experimental sets. After incubation at 37°C, absorbance was noted using UV/Visible spectrophotometer. The maximum activity and concentration of secondary metabolite by *A. niger* was observed at pH 5.5, 1.5 ml substrate concentration and 5ml inoculum concentration which inhibited the bacterial growth up to 0.079 OD (Table 1). The contour plots showing the interacting effect between pH vs substrate, pH vs inoculum concentration and substrate vs inoculum concentration for the production of antimicrobial agents have been shown in Figure 3A, B, C). The ANOVA table and R^2 values of various factors showed that RSM design was significant (Table 2). Similar findings have been observed by other researchers that physiological conditions have an overall effect on production of secondary metabolites as antimicrobial agents (Chandrakar and Gupta, 2018).

Table 1: RSM approach for optimizing maximum production of antimicrobial agents as secondary metabolite from *Aspergillus niger*. The bacterial biomass after inhibition through antimicrobial agents, was noted at 600 nm using UV/Visible spectrophotometer. The least biomass was observed in trial No. 12.

Sr. No.	pH	Substrate (mL)	Inoculum Conc. (mL)	OD_{600nm} (Mean±SD)
1	3	3	8	0.379±0.8
2	5.5	9	5	0.486±0.8
3	5.5	9	5	0.242±0.8
4	8	3	2	0.377±0.8
5	8	15	8	0.488±0.8
6	3	15	2	0.252±0.8
7	8	3	8	0.258±0.8
8	3	15	8	0.263±0.8
9	8	15	2	0.339±0.8
10	3	3	2	0.374±0.8
11	5.5	19	5	0.308±0.8
12	5.5	1.5	5	0.079±0.8
13	2	9	5	0.308±0.8
14	5.5	9	1	0.551±0.8
15	9.5	9	15	0.517±0.8
16	5.5	9	10	0.355±0.8
17	5.5	9	5	0.432±0.8
18	5.5	9	5	0.441±0.8
19	5.5	9	5	0.446±0.8
20	5.5	9	5	0.442±0.8

Antibiotic test

After optimization of various parameters for increasing the production of antibiotic as secondary metabolite, the ability of produced inhibitor to inhibit growth of bacterial strains was checked. The selected strains included *Streptococcus aglactaie* causing urinary tract infection, *Streptococcus pyogene* causing skin infections, *Escherichia coli* causing intestinal infection and *Proteus mirabilis* causing pneumonia. The sensitivity was checked through measuring the zone of inhibition for each strain. The zone of inhibition for *E. coli*, *S. Pyogne*, *P. Mirabilis* and *S. agalactiae* was 18,

53, 14 and 20 mm respectively. As per finding, our method of disc diffusion assay was more reliable in comparison to other methods for potential screening of microorganisms producing antibiotic compounds and this is in accordance with the previously reported assays (Buzón-Durán *et al.*, 2018).

Table 2: ANOVA table describing the interaction of various factors for production of antimicrobial agents.

Term	Effect	Coef	SE Coef	T-value	P-value
Constant		0.4287	0.0226	18.99	0.000
pH	0.2518	0.1259	0.0364	3.46	0.006
Substrate (mL)	0.1732	0.0866	0.0346	2.50	0.031
Inoculum Conc. (mL)	-0.0886	-0.0443	0.0483	-0.92	0.381
pH*pH	-0.0404	-0.0202	0.0486	-0.42	0.686
Substrate (mL)*Substrate (mL)	-0.4856	-0.2428	0.0363	-6.69	0.000
Inoculum Conc. (mL) *Inoculum Conc. (mL)	0.0930	0.0465	0.0605	0.77	0.460
pH*Substrate (mL)	0.1969	0.0984	0.0398	2.47	0.033
pH*Inoculum Conc. (mL)	0.0679	0.0339	0.0558	0.61	0.557
Substrate (mL)*Inoculum Conc. (mL)	0.2926	0.1463	0.0619	2.36	0.040

R^2 = 90.70 %.

Molecular characterization

Molecular characterization was performed in order to confirm the identification of *A. niger* (Iwen *et al.*, 2002). After sequencing it was confirmed that strain was *Aspergillus niger* and sequence has been submitted in NCBI GenBank for allotment of accession number.

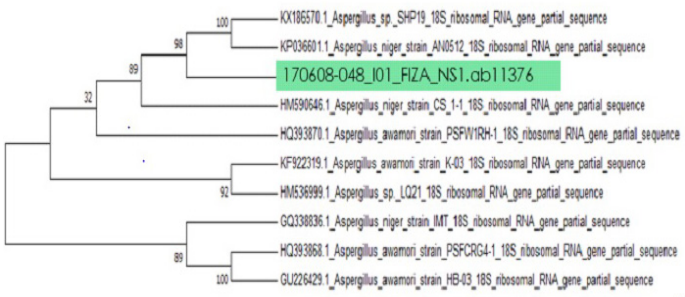

Figure 2: Phylogenetic tree of isolated strain of *Aspergillus niger*. The branching pattern was generated using neighbour joining method. The organism along with their gene bank accession number are shown at the end of each node. Newly identified sequence showed 98% homology with *Aspergillus niger* strains.

Phylogenetic analysis

Phylogenetic tree was constructed using Basic Local Alignment Search Tool (BLAST) against 18s rRNA gene of locally isolated *A. niger* and its relationship with other strains was observed (Hamid *et al.*, 2018). Top ten highly related sequences were selected for constructing phylogenetic tree via MEGA6 on the bases of e-score (Fig.2).

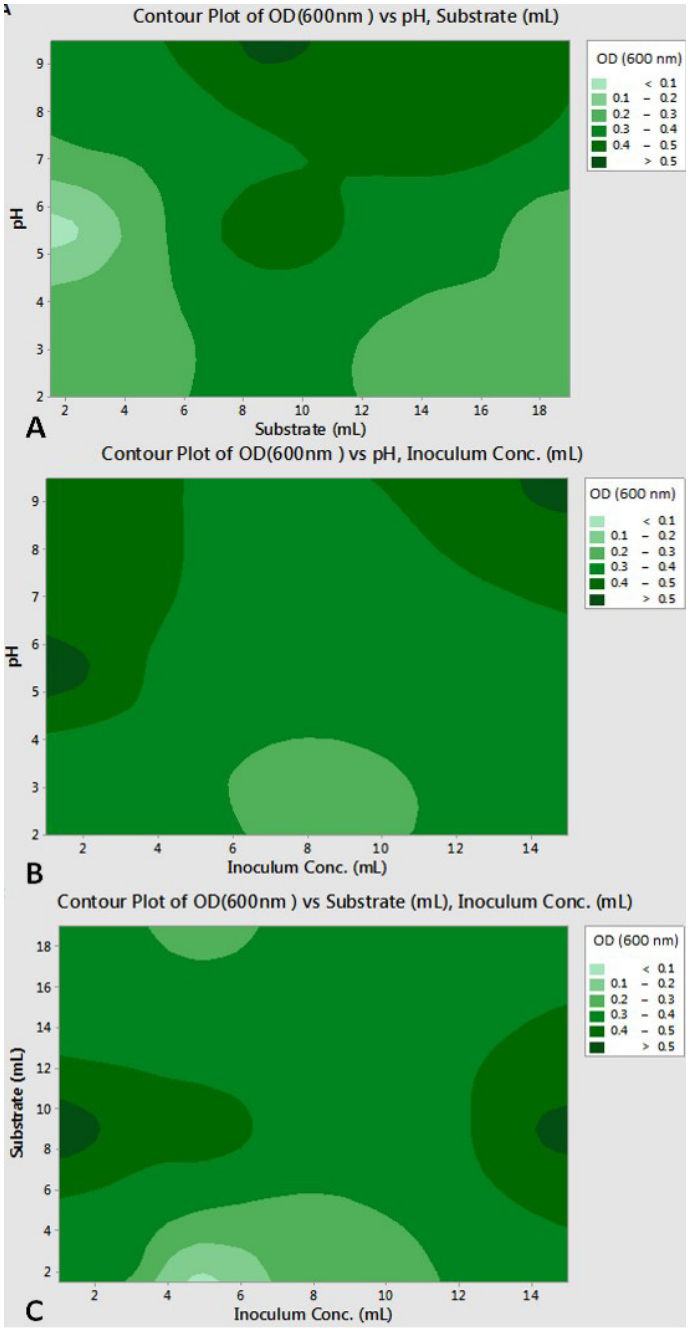

Figure 3: Interaction of pH vs substrate (A), pH vs inoculum concentration (B) and substrate vs inoculum concentration (C).

Prediction of secondary metabolite gene clusters

Natural Product Domain Seeker (NaPDoSR) was employed for analyzing the sequence specific domains which are involved in the formation of secondary metabolite. For this purpose, gene sequence was extracted, trimmed and translated. The sequence was subjected to domain specific phylogenetic clustering and possible products of gene were investigated. Six KS and 5 C-domain within sequence was predicted by software. For further conformation, antiSMASH was also employed to get authenticity of the results which also confirmed presence of secondary

metabolite genes within the sequence. The similarity of genes was observed to elucidate relational similarity of biosynthetic pathways to produce similar compounds (El-Moneam *et al*., 2017). Four different biosynthetic pathways and five types of secondary metabolite were identified during comparison of gene.

Determination of features of gene clusters and possible metabolites

Results of NaPdoSR were further authenticated through antiSMASH tool, as these predicted presence or absence of secondary metabolite synthesizing domains within the sequence. This tool comprehensively briefed clusters of gene that were involved in the formation of secondary metabolite by using comparative approach with the other sequences available in database having annotated gene clusters (Palazzotto and Weber, 2018). Seven gene clusters were observed (four with complete annotation), capable of forming secondary metabolite. Among these, three clusters belonged to Aflatrem, Monacolin K and Fusarubin without any reported inhibitory effect while the fourth one Tautomycetin has reported inhibitory effect against serine/threonine phosphatses which might be due to its effects on phosphatase pathways of bacterial strain leading to hindering the bacterial growth (El-Moneam *et al*., 2017).

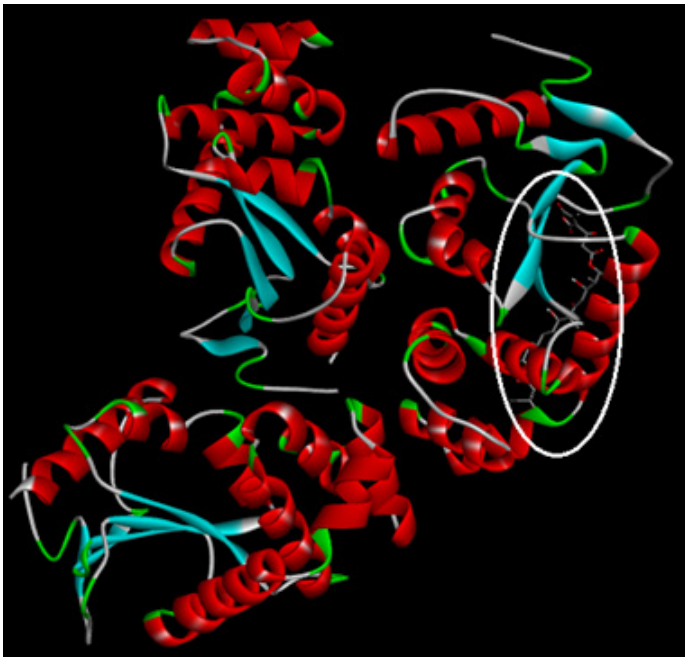

Figure 4: Interaction of Tautomycetin with *Proteus mirabilis* via Auto dock Vina. It was further observed that complex structure of *P. mirablis* DsbA (C30S) with a non-covalently bound peptide PWATCDS (4OD7) was also inhibited by tautomycetin leading to bacterial cell lysis.

Docking analysis

Docking analysis was performed to determine the interactions of tautomycetin inhibitors with bacterial strains i.e *S. Pyogene, S. Aglactaiae, E. coli* and *P. Mirabilis.* It was observed that Tautomycetin possessed anticancer and antibacterial activities by inhibiting protein phosphatases like PP1, PP2A and SHP2 (Shoda *et al*., 2017). Tautomycetin inhibitor interacts with crystal structure of unsaturated glucuronyl hydrolase from *S. agalactiae* (3ANJ) leading to bacterial death (Packiavathy *et al*., 2012). Tyrosine phosphatase of *E. coli* (2FEK) was inhibited by tautomycetin. It was further observed that complex structure of *P. mirablis* DsbA (C30S) with a non-covalently bound peptide PWATCDS (4OD7) was also inhibited by tautomycetin leading to bacterial cell lysis (Fig. 4). The mode of inhibition of *S. Pyogene* by tautomycetin has been still unidentified because of incomplete sequence availability.

Conclusion

Four putative indigenous fungal strains *R. stonolifer*, *A. niger*, *A. fumigatus* and *A. terreus* were isolated from different regions of Pakistan and these strains were capable of producing antimicrobial compounds as their secondary metabolites. Among these strains, *A. niger* was found best for the production of antimicrobial agent Tautomycetin using banana peels through fermentation as it was secreted in maximum amount as secondary metabolite. The results were confirmed by docking analysis to analyze computational interactions and it was observed that *S. aglactaie*, *E. coli* and *P. mirabilis* had interaction with tautomycetin while *S. pyogene* did not show any interaction. Tautomycetin has been reported to inhibit the growth of *S. pyogene* and there is further need to investigate the mode of antibacterial activity by tautomycetin.

Disclosure of potential conflict of interest

The authors declare that they have no conflict of interest.

Compliance with ethical standards

This research follows all ethical standards and do not involve human and animal trials.

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