IBBJ

Autumn 2017, Vol 3, No 4

Original Article

Optimization of Antibacterial Compounds Production by *Aspergillus fumigatus* Isolated from Sudanese Indigenous Soil

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Submitted 5 May 2017; Accepted 22 Jun 2017; Published 30 Jul 2017

The purposes of the present study were to screen the ability of *Aspergillus fumigatus* to produce antibacterial compounds using different growth parameters namely, pH, temperature, agitation and time of fermentation and investigate the effect of the yield against bacterial isolates. *A. fumigatus* was isolated from arable lands in Sudan and identified macroscopically and microscopically. The pure fungal culture was inoculated in fermentation medium with different growth parameters. The fungal metabolites were extracted in intervals of 7, 8, and 9 days incubation period. No yield was observed at pH values of 6 and 8. A relatively high yield of active compounds was observed at pH 7, agitation speed of 75 rpm and 7 days contact time, while the highest yield was exhibited at pH 7, agitation speed of 100 rpm and 8 days contact time. These metabolites were investigated for their inhibitory effects against indicator bacterial species. All indicator bacterial species showed resistance to 2.5% concentration of the crude extract. The 5% crude extract concentration exhibited small inhibitory effect (8 mm) against *Salmonella typhimurium*, moderate inhibitory effect (19 mm) against *Listeria monocytogenes* and high inhibitory effect (25 mm) against *Pseudomonas aeruginosa*. The pronounced inhibitory effect was observed with 10% concentration against *Pseudomonas aeruginosa* (40 mm) followed by *Listeria monocytogenes* (30 mm). A moderate inhibitory effect of the 10% crude extract was observed with *Salmonella typhimurium* (13 mm). The indicator bacteria *Staphylococcus aureus* showed resistance to all tested concentrations. The present study show that the growth parameters affect the yield but not the viability of *Aspergillus fumigatus*. The fungal metabolites have antibacterial effects.

Keywords: *Aspergillus fumigatus*, optimization, antibacterial activity

Natural products are important sources in the drug discovery process. Accordingly, new ecological niches should be explored for natural bioactive agents in pharmaceutical, agricultural, and industrial fields. These products should be renewable, ecofriendly and easily obtainable. The most prominent producers of natural products are plants, animals, marine microorganisms (sponge, corals and algae), and microorganisms (bacteria, actinomycetes, and fungi) (1).

The presence of more than 200,000 natural metabolites presenting various bioactive properties (2) demonstrates the importance of natural products in new drugs discovery.

The use of microorganisms has created a huge revolution in many aspects of human's life as

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witnessed by numerous studies conducted by scientists in different fields. After identifying microorganisms, humans learned to control their undesirable properties (3). Microorganisms are used in production of pharmaceutical products, especially antibiotics (4).

Antibiotics have an important role in human health. Their necessity emerged from the spread of various diseases. As a result, scientists are trying to produce and discover more antibiotics (5).

Soil fungi have been the most studied of fungi, and typical soil genera such as *Acremonium*, *Aspergillus*, *Fusarium* and *Penicillium* have shown ability to synthesize a diverse range of bioactive compounds. About one third of metabolites derived from fungi belong to *Aspergillus* and *Penicillium* (2).

Aspergillus fumigatus is a ubiquitous saprophytic fungus, and is involved in environmental carbon and nitrogen recycling. It can also cause severe fatal invasive aspergillosis in immunocompromised patients with a widespread occurrence (6). On the other hand, some strains of *A. fumigatus* can produce metabolites with a wide range of biological activities (7). Different *Aspergillus* strains can produce a large variety of secondary metabolites under different environmental conditions. Modifying fermentation parameters such as time, temperature, pH, and nutrients can help expanding the range of those secondary metabolites (8). The presence of antimicrobial properties may indicate a larger activity spectrum, including antitumor and antiparasitic characteristics (9).

The purpose of the present study was to screen the ability of *Aspergillus fumigatus* to produce antibacterial compounds using different growth parameters namely, pH, temperature, agitation, and time of fermentation, and investigate the effect of the yield against four bacterial isolates.

Materials and methods

Study design

This experimental cross sectional case study was conducted in four different regions in Sudan known as Shandy (River Nile state), Almosalamia (Aljazeera state), Madani (Aljazeera state), and Fadasy (Aljazeera state). These area were selected for their diversity.

Samples collection

Eight soil samples from arable lands were collected in sterile plastic bags. All experiments were conducted aseptically in the microbiology laboratory (10).

Isolation of thermophilic *Aspergillus*

One gram soil sample was taken and suspended into 10 ml sterile distilled water. An amount of 1 ml soil suspension was taken and poured into sterilized potato dextrose agar (PDA) medium. All inoculated plates were incubated at 45 °C for 7 days. Selected fungal hyphae were picked and purified by sub-culturing on PDA plates. After the incubation period, smear from culture was stained by lactophenol cotton blue stain and examined microscopically using 10x and 40x objective lenses (11).

Production of fungal biomass

An amount of 250 ml synthetic broth medium (5 g/l yeast extract as (nitrogen source), 1 g/l KH_2PO_4, 0.5 g/l $MgSO_4.7\ H_2O$, 0.01 g/l $FeSO_4.7\ H_2O$, 15 g/l NaCl, 30 g/l starch as (carbon source)), was prepared in Erlenmeyer flasks (500 ml capacity) and sterilized at 121 °C, 15 Ibs for 15 min. After sterilization, the medium was set to cool at room temperature, and was inoculated by purified *A. fumigatus* which was prepared by suspending spores harvested with 0.1% Tween 80 from a three day old PDA culture (12).

Optimization process

The optimum conditions such as incubation period, pH, temperature, and agitation rate used for the production of secondary metabolites were studied. Three flasks containing synthetic broth medium were taken, inoculated with test microorganisms, labeled as pH 6, 7, 8 and incubated for 7, 8, and 9 days aerobically. Each preparation at specific pH value was incubated in triplicate at 20

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°C, room temperature, and 40 °C. The three incubated flasks at room temperature were agitated at 50 rpm, 75 rpm, and 100 rpm for the entire incubation durations (13).

Extraction of fungal metabolites

After 7, 8, and 9 days incubation period, 20 ml of inoculated media were transferred into falcon tubes. The falcon tubes were centrifuged using a cold centrifuge device at 5000 rpm for 40 min to separate the fungal mycelium from the supernatant. Equal ratios (l/1) of acetonitrile organic solvent and crude extract were mixed, centrifuged at 5000 rpm for 40 min at 4 °C, and the crude solvents were collected. This step was repeated 3 times. The substrate solvent mixture was allowed to become thoroughly wet in the refrigerator for 2 h followed by 2 h of mechanical agitation, and then it was filtered using filter paper. The filtrate was dried using water bath at 45 °C with a slight rotation of 100 rpm. The extract was taken into 10 ml of solvent and kept at 4 °C before using for antimicrobial activity test (14).

Antimicrobial sensitivity testing

One gram of crude extract was dissolved in 10 ml distilled water (10% concentration). Then 1 ml aliquot was taken and added to another test tube containing 1 ml distilled water to obtain 5% concentration. This step was repeated to obtain 2.5% concentration. The pieces of filter paper were punctured and immersed in 0, 5 and 10% crude extracts, transferred to Petri plates containing Muller-Hinton agar medium inoculated with different species of test microorganisms. These included two-Gram negative bacteria (*Pseudomonas aeruginosa, Salmonella typhymirium*) and two-Gram positive bacteria (*Listeria monocytogenes, Staphylococcus aureus*). The Petri dishes were pre-incubated at 4 °C for 2 h to allow uniform diffusion of crude extracts onto the agar. After pre-incubation, the plates were incubated for 24 h at 37 °C. The diameter of the clear zone around each disc was measured and compared with control agar plates containing discs with solvent only (negative control) (15).

Results

Identification of the thermophilic *Aspergillus fumigatus*

Various numbers of fungi were isolated using PDA, but *Aspergillus fumigatus* colonies color changed with the incubation time. It was firstly white, blue- green and thereafter green dark to blackish- gray after 7 days in PDA plates. The diameter of the colonies varied from 18 to 50 mm after the fifth day. The fungus produced superficial and submerged hyphae on PDA medium; however their texture was velvety, denser in the center figure 1.

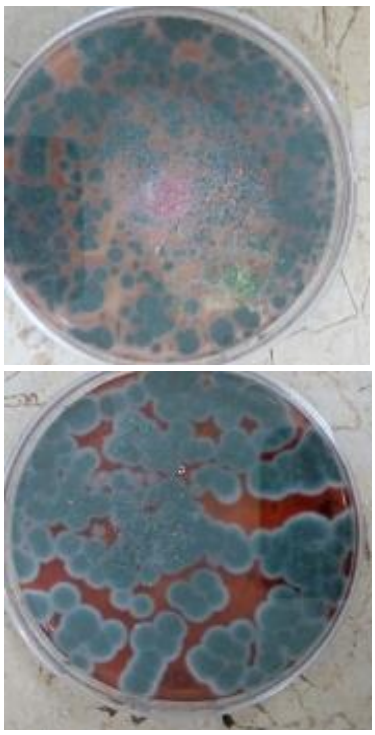

Figure 1. Colony morphology of *Aspergillus fumagatus* in PDA media.

Isolate showed numerous round and refractive conidia. The hyphae were septate and hyaline. Conidial heads were uniseriate with compact columnar. Conidiophores were simple, uncolored and smooth terminating in a globose swelling. Conidia were globose to ellipsoidal, green with 2.5 to 3 µm diameter. Based on these typical features, the isolate has been identified to belong to the

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species *Aspergillus fumigatus* figure 2.

Optimization of culture conditions for higher production of antimicrobial compounds

As shown in table 1, the yield of active compounds that inhibited the growth of test bacterial species was negative for extracts of fungi grown at pH of 6 and 8 The high yield of active compounds was observed for extracts of fungi grown at pH 7, agitation speed of 75 rpm and 7 days contact time, while the highest one was exhibited at pH 7, agitation speed of 100 rpm and 8 days contact time.

Antibacterial activity of *Aspergillus fumiatus* metabolites of crude extract against indicator bacterial species

The 2.5% crude extract showed no effect against all indicator bacterial species tested. The 5% crude extract exhibited small inhibitory effects (8 mm) against *Salmonella typhimurium*, moderate inhibitory effect (19 mm) against *Listeria monocytogenes*, and high inhibitory effect (25 mm) against *Pseudomonas aeruginosa*. The pronounced inhibitory effect was demonstrated at 10% concentration (40 mm) against *Pseudomonas aeruginosa* followed by (30 mm) against *Listeria monocytogenes*. The moderate inhibitory effect of 10% concentration (13 mm) was observed with *Salmonella typhimurium*. While indicator bacteria *Staphylococcus aureus* showed resistance to all tested concentrations.

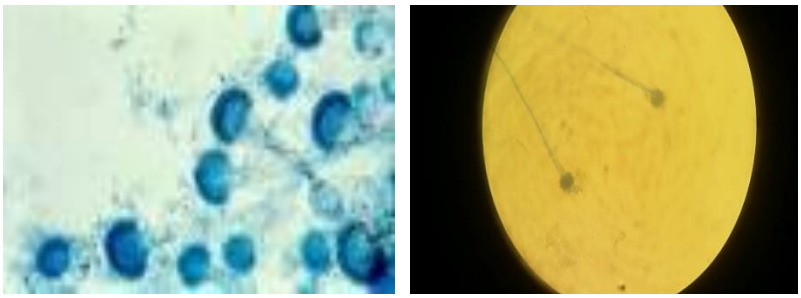

Figure 2. Microscopic view (40x) of *Aspergillus fumagatus*.

Table 1. The effect of culture conditions for higher production of antimicrobial compounds qualitatively

Yield of active compound	pH value			Temperature (°C)			Agitation (rpm)			Contact time (day)		
	6	7	8	20	Room	40	50	75	100	7	8	9
Zero	√	-	-	√	√	√	√	√	√	√	√	√
Positive	-	√	-	-	√	-	-	√*	√**	√*	√**	-
Zero	-	-	√	√	√	√	√	√	√	√	√	√

√: selected parameter; √*: small inhibition zone; √**: large inhibition zone.

Discussion

The culture characteristics of isolated fungus were in agreement with Hamidou *et al*. (16), who reported that the fungus grew well even up to 37 °C on PDA medium; however their texture was velvety, and denser in the center. The yield of active compounds inhibiting the growth of test bacterial species was negative at both pH values of 6 and 8, which correlated with other parameters. The high yield of active compounds was observed at pH 7, agitation speed of 75 rpm and 7 days contact time. This result is in agreement with Lamrani *et al*. (17), who reported the production of fumagillin and helvolic acid from *Aspergillus fumigatus* after 6 days of incubation, and production of fumagillin after 7 days on millet wheat medium by *Aspergillus*

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fumigatus strain isolated from trituration units in Morocco, while the highest yield was exhibited at pH 7, which is also in agreement with the results of Hamidou *et al*. (16), who found that the maximum growth, as well as highest antimicrobial activity by *Aspergillus fumigatus* isolate were achieved at pH 7, but a sensible variation of the pH reduced significantly the production of metabolite compounds. In the present study, the highest antimicrobial yield was obtained at 100 rpm agitation speed and 8 days contact time which is in disagreement with Liu *et al*. (1). The *Aspergillus fumigatus* was grown in all pH values, agitation speeds, and contact times but the production of its metabolites was affected by varies parameters. In our study the fungus metabolites were better produced at room temperature. This result is in disagreement with Hamidou *et al*. (16) and Boudra and Morgavi (18), who incubated Erlenmeyer flasks at 37 °C to produce fumagillin and gliotoxin by *Aspergillus fumigatus* isolated from Sweden and France, respectively. The 2.5% concentration showed no effect against all indicator bacterial species. The highest inhibitory effect of secondary metabolites produced by *A. fumigatus* was 40 mm/ 10% concentration against *Pseudomonas aeruginosa* followed by 30 mm / 10% concentration against *Listeria monocytogenes*. This is in line with Hamidou *et al*. (16) who reported the higher inhibition zone against *Pseudomonas aeruginosa*, followed by *Listeria monocytogenes*.

Also these findings are similar to those found by Takahashi *et al*. (19) who reported antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Streptococcus pyogenes*, and *Listeria monocytogenes*.

In conclusion, the present study was successful in producing antimicrobial compounds by a simple, non-aseptic, and low-cost single process used for isolating thermophilic *Aspergillus fumigatus* from soils in different areas of Sudan. The optimization process carried out in the synthetic broth, showed that the optimum incubation period for the production of antimicrobial compounds was 8 days, at pH 7, and room temperature with 100 rpm shaking speed.

Conflict of interest

The authors declared no conflict of interest.

References

1. Liu J Y, Song Y C, Zhang Z, et al. Aspergillus fumigatus CY018, an endophytic fungus in Cynodon dactylon as a versatile producer of new and bioactive metabolites. J Biotechnol. 2004;114:279-87.

2. Berdy J. Recent developments of antibiotic research and classification of antibiotics according to chemical structure. Adv Appl Microbiol. 1974;18:309-406.

3. Asnaashari M, Ghanbary M A T, Tazick Z. Optimization of penicillin G production by Penicillium chrysogenum. Ann Biol Res. 2012;3:5434-40.

4. Philippe P, Esther M, Lucas M, et al. Novel antimicrobial secondary metabolites from a Penicillium sp. isolated from Brazilian cerrado soil. 12. Electronic J Biotechnol. 2009;12:1-9.

5. Rolain J M, Maurin M, Raoult D. Bactericidal effect of antibiotics on Bartonella and Brucella spp.: clinical implications. J Antimicrob Chemother. 2000;46:811-4.

6. Latge J P. Aspergillus fumigatus and aspergillosis. Clin Microbiol Rev. 1999;12:310-50.

7. Son K-H, Kim Y-K, Lee H-W, et al. Amplification of farnesyl protein transferase inhibitory activity from Aspergillus fumigatus F93 by Plackett-Burman design. Biotechnology Letters. 1996;18:1297-300.

8. Pfefferle C, Theobald U, Gurtler H, et al. Improved secondary metabolite production in the genus Streptosporangium by optimization of the fermentation conditions. J Biotechnol. 2000;80:135-42.

9. Demain A L. Pharmaceutically active secondary metabolites of microorganisms. Appl Microbiol Biotechnol. 1999;52:455-63.

10. Cheesbrough M. Medical laboratory manual for tropical countries Volume II. Microbiology 1981; 264-390.

11. Aneja KR. Experiments in Microbiology Plant Pathology and Biotechnology, Fourth Edition, New Age International (P) Ltd., Publishers, New Delhi, 2003;438-41.

12. Zhang L, Wang S-Q, Li X-J, et al. New insight into the stereochemistry of botryosphaeridione from a Phoma endophyte. J Mol Struct. 2012;1016:72-5.

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13. Kalic R, Sallet D, Kuhn R C, et al. Optimization of fermentation media for the growth of a fungus used as bioherbicide. Congresso Brasileir de Engenharia Quimica. 2014:1-8.

14. Kumar S, Kaushik N, Proksch P. Identification of antifungal principle in the solvent extract of an endophytic fungus Chaetomium globosum from Withania somnifera. SpringerPlus. 2013;2:37.

15. Lalitha M. Manual on antimicrobial susceptibility testing. Performance standards for antimicrobial testing: Twelfth Informational Supplement. 2004; 454-6.

16. Hamidou C H, Sawadogo-Lingani H, Savadogo A. Isolement et caractérisation morphologique de moisissures productrices de substances antibactériennes à partir d'aliments locaux au Burkina Faso. Int J Biol Chem Sci. 2016;10:198-210 (Article in French).

17. Lamrani K, Lakhtar H, Ismaili Alaoui M. Production of fumagillin by Aspergillus fumigatus isolated from traditional trituration units, "Maasra", in Morocco. Micologia Aplicada International. 2008;20:35-41.

18. Boudra H, Morgavi D P. Mycotoxin risk evaluation in feeds contaminated by Aspergillus fumigatus. Anim Feed Sci Technol. 2005;120:113-23.

19. Takahashi J A, Castro M C M d, Souza G G, et al. Isolation and screening of fungal species isolated from Brazilian cerrado soil for antibacterial activity against Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Streptococcus pyogenes and Listeria monocytogenes. J Mycol Med. 2008;18:198-204.

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