International Journal of Modern Agriculture, Volume 10, No.2, 2021
ISSN: 2305-7246

Antimicrobial activity of Secondary Metabolites of fungi isolated from soil against selected pathogenic bacteria

Nadia Mubarik[1], Ghadir Ali[2], Muhammad Khrram[2], Aneeqa Sadiq[3], Ijaz Ahmad[4], Sidra Iqbal[4], Salman Hussain[5], Adnan Hussain Tanoli[6], Mir Sadiq Shah*[7], Muneeba Wali[8]

[1]Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar

[2]School of Science, Department of Life Sciences, University of Management and Technology, Lahore, Pakistan

[3]Department of Microbiology and Molecular genetics, The Women University, Multan

[4]Department of Allied Health Sciences, Superior University Lahore

[5]DHQ Hospital South City Okara, Primary and Secondary Healthcare Department

[6]Department of Microbiology, University of Haripur, KPK Pakistan

[7]Department of Zoology, University of Science and Technology Bannu, Bannu-28100, Khyber Pakhtunkhwa, Pakistan

[8]Department of Allied Health Sciences, Institute of Integrative Biosciences, CECOS University Pakistan.

Corresponding Author: Muneeba.wali@gmail.com

Abstract

Background: Due to the rise of drug-resistant bacteria, the need for novel antibiotics is growing by the day. Despite the abundance of possible antibiotic sources, such as medicinal plants, soil remains the most significant reservoir for new antibiotics with pharmacological and biological action.

Objective: The goal of this research was isolation and identification of soil fungus, as well as to determine their antimicrobial activity against pathogenic microorganism.

Methodology

Soil samples were taken at several locations within the district of Mardan. The fungal isolates were sampled and isolated using the direct inoculation technique. Each fungal isolate was microscopically examined for identification. Secondary metabolites were extracted and chromatographic analysis was performed on all of the fungal extracts. The disc-diffusion technique was used to determine the antibacterial activity of secondary metabolites produced by isolated fungal strains according to previous study.

Results: In our study, *Aspergillus fumigates* was isolated from soil samples. The various phytochemicals observed in *A. fumigatus* were tannins, flavonoids and terpenoids. 100 µL concentration of the metabolite was used for antimicrobial activity. The zone of inhibition against *Pseudomonas aeruginosa, E.coli, Staphylococcus aureus* and *Salmonella typhi* were 19 mm, 20 mm, 18 mm, 19 mm respectively.

Conclusion: The substantial genetic diversity identified in *A. fumigatus* strains might lead to the discovery of novel metabolites that could be used to generate new antibiotics.

Key words: Antimicrobial activity; Secondary Metabolites; Pathogenic bacteria

Introduction

Antibiotics are biologically derived natural substances used to cure disease caused by microorganisms in eukaryotes such as humans (1). Antibiotics are classified as wide-spectrum or

narrow-spectrum depending on how they work. Inhibition of cell walls, proteins, and nucleic acids are among them (2). Due to the rise of drug-resistant bacteria, the need for novel antibiotics is growing by the day. Despite the abundance of possible antibiotic sources, such as medicinal plants, soil remains the most significant reservoir for new antibiotics with pharmacological and biological action (3). Every year, more than 500 new antibiotics are discovered, yet over 60% of them come from the soil (4). Unexpectedly, a few grams of soil may contain a large number of microorganisms (5).

Penicillium, Fusarium, Aspergillus, Cladosporium, and Yeasts are among the fungi that may synthesize enzymes and secondary metabolites such as antibiotics (6). Antibiotics derived from soil-isolated fungus account for around 20% of the total (7). Antibiotics generated by fungi, such as fusidic acid (8), cephalosporin, and penicillin, are extensively utilized for the treatment of a broad range of disorders (9) and it is the most significant source of potentially significant pharmaceutical medications (10). More than 98,000 fungus species have been identified to date (11). These are just a small percentage of the total number of fungal species on the planet, which is predicted to be around 0.6 and 9.9 million (11). The challenges faced while growing filamentous fungus in labs might partially explain the low discovery rate of fungal species. More than 99 percent of the microorganisms found in soil samples have been reported to be unable to culture in a lab setting (12). This clearly shows that current natural product separation methods do not fully use known microbial biosynthetic capabilities. As a result, there is still a sufficient space for microbes to be studied in order to discover novel secondary metabolites. The goal of this research was isolation and identification of soil fungus, as well as to determine their antimicrobial activity against pathogenic microorganism.

Materials and methods

Isolation of fungi from soil

Soil samples were taken at several locations within the district of Mardan. The fungal isolates were sampled and isolated using the direct inoculation technique. Soil samples were put individually onto surface malt agar medium and incubated at 25 °C for 5–7 days. For isolation of all fungi Sabouraud dextrose agar was used. Al-Enazi et al. described the approach that was applied in this study (13).

Fungal isolates identification

Each fungal isolate was examined both microscopically and macroscopically according to previous study (14).

Secondary metabolites extraction

Two liters of n-butanol (saturated with water) were used to extract one liter of each fungal growth medium, which was then re-extracted four times. A Buchner funnel containing anhydrous sodium sulphate was used to filter the resulting butanol extract. The butanol extracts were collected and dried from the solvent using a rotatory evaporator at a temperature of not more than 35°C. The dry extract was then stored in the fridge for further analysis (15). Chromatographic analysis was performed on all of the fungal extracts. Before and after spraying with Antimony trichloride, the spots were seen under UV light ($SbCl_3$).

Antimicrobial activity

The test organisms used were *Pseudomonas aeruginosa*, *E.coli*, *Staphylococcus aureus* and *Salmonella typhi*. The disc-diffusion technique was used to determine the antibacterial activity of secondary metabolites produced by isolated fungal strains according to previous study (13). Test organisms were grown in petri dishes containing 20 mL of agar media. Standard discs (6 mm in diameter) containing 100µl of fungal extract were put on the agar and incubated for 24–48 hours at 37 °C. The diameter of the inhibition zone produced around the disc was used to measure antibacterial activity.

Results and discussion

In our study, *Aspergillus fumigates* was isolated from soil samples. A previous study done by Raja et al. collected different soil sample for isolation and identification of various strains of fungi in India (16). It was also hypothesized that the quantity and frequency of fungus species isolated from soil are influenced by the soil's moisture content and/or organic carbon content (17).

The various phytochemicals observed in *A. fumigatus* were tannins, flavonoids and terpenoids. Antimicrobial activity of these metabolites was checked against *Pseudomonas aeruginosa*, *E.coli*, *Staphylococcus aureus* and *Salmonella typhi* by using well diffusion method. 100 µL concentration of the metabolite was used for antimicrobial activity. Maximum zone of inhibition were reported at different concentration of different bacteria. Variation in the zone of inhibition was observed against different bacteria. The zone of inhibition against *Pseudomonas aeruginosa*, *E.coli, Staphylococcus aureus* and *Salmonella typhi* were 19 mm, 20 mm, 18 mm, 19 mm respectively. An earlier study reported comparable results to our findings (2). Numerous investigations on the antibacterial activity of fungi and their secondary metabolites have shown that the antimicrobial activity is dependent on the secondary metabolites nature present in the fungus (8, 18). The fungal producers may be able to change the nature of the end synthesized products depending on the environmental circumstances, and a huge number of bioactive compounds may be discovered after optimizing both growth and production settings. Previously, it was found that the addition of pool of bacteria to an *A. fumigatus* culture boosted the synthesis of several metabolites (19). The substantial genetic diversity identified in A. fumigatus strains might lead to the discovery of novel metabolites that could be used to generate new antibiotics.

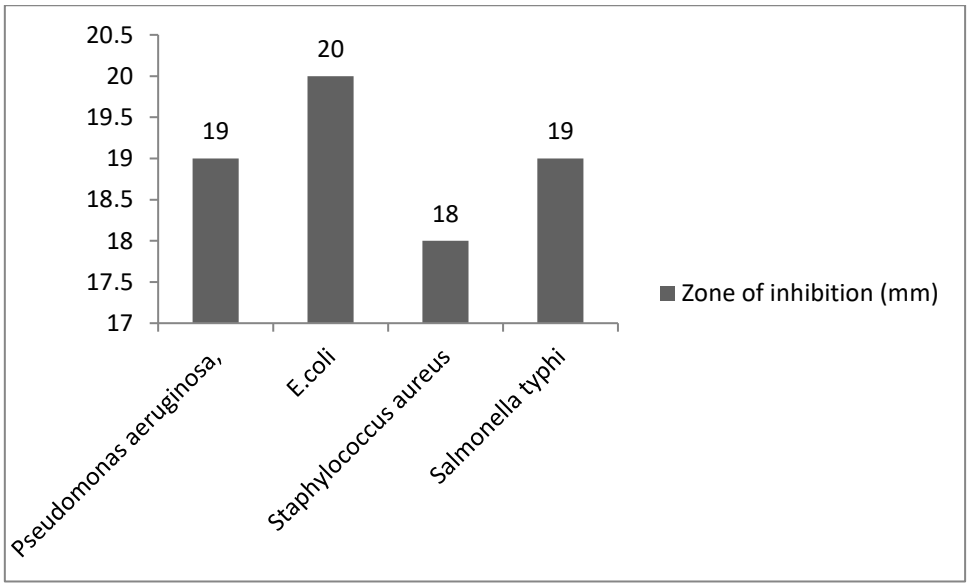

Figure 1: Antimicrobial activity of secondary metabolites against different pathogens

References

1. Pannapa P, Pattra S. Antimicrobial and enzyme activity produced by Bacillus Spp. Isolated from soil. Int J Pharm Pharm Sci. 2017;9(3):205-10.
2. Alkhulaifi MM, Awaad AS, Al-Mudhayyif HA, Alothman MR, Alqasoumi SI, Zain SM. Evaluation of antimicrobial activity of secondary metabolites of fungi isolated from Sultanate Oman soil. Saudi Pharmaceutical Journal. 2019;27(3):401-5.
3. Rajaperumal S, Nimmi M, Kumari BR. In vitro studies on antimicrobial and antioxidant effect of methanolic extract of Indigofera aspalathoides (Vahl ex DC) and its cytotoxic property against human lung cancer cell line NCI H460. Eur J Exp Biol. 2013;3(3):18-29.
4. Molinari G. Natural products in drug discovery: present status and perspectives. Pharm Biotechnol. 2009:13-27.
5. Makut M, Owolewa O. Antibiotic-producing fungi present in the soil environment of Keffi metropolis, Nasarawa state, Nigeria. eubacteria. 2011;10(18):19.
6. Hyde K. Increasing the likelihood of novel compound discovery from filamentous fungi. Bio-exploitation of filamentous fungi. 2001:77-91.
7. Berdy J. Bioactive metabolites, a personal view. J Antibot. 2005;58(1):1-26.
8. Akpotu MO, Eze PM, Abba CC, Nwachukwu CU, Okoye FB, Esimone CO. Antimicrobial activities of secondary metabolites of endophytic fungi isolated from Catharanthus roseus. Journal of Health Sciences. 2017;7(1):15-22.
9. Sethi S, Kumar R, Gupta S. Antibiotic production by microbes isolated from soil. International Journal of Pharmaceutical Sciences and Research. 2013;4(8):2967.
10. Wasser S. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol. 2002;60(3):258-74.
11. Scheffers BR, Joppa LN, Pimm SL, Laurance WF. What we know and don't know about Earth's missing biodiversity. Trends Ecol Evol. 2012;27(9):501-10.
12. Torsvik V, Øvreås L. Microbial diversity and function in soil: from genes to ecosystems. Curr Opin Microbiol. 2002;5(3):240-5.
13. Al-Enazi NM, Awaad AS, Al-Othman MR, Al-Anazi NK, Alqasoumi SI. Isolation, identification and anti-candidal activity of filamentous fungi from Saudi Arabia soil. Saudi Pharmaceutical Journal. 2018;26(2):253-7.
14. Casero M, Ascaso C, DiRuggiero J, Meslier V, Artieda O, Quesada A, et al., editors. Biogeography at microscopic scale: diversity of endolithic microbial communities in icrohabitats of gypcrete from the Atacama Desert. Book of Abstracts VII International Conference on nvironmental Industrial and Applied Microbiology–BMW2017 Madrid (Spain); 2017.
15. Zain ME, Awaad AS, Al-Outhman MR, El-Meligy RM. Antimicrobial activities of Saudi Arabian desert plants. Phytopharmacology. 2012;2(1):106-13.
16. Raja M, Praveena G, William SJ. Isolation and identification of fungi from soil in Loyola college campus, Chennai, India. Int J Curr Microbiol App Sci. 2017;6(2):1789-95.
17. Salar RK, Aneja K. Thermophilous fungi from temperate soils of northern India. Journal of Agricultural Technology. 2006;2(1):49-58.
18. Wang X, Radwan MM, Taráwneh AH, Gao J, Wedge DE, Rosa LH, et al. Antifungal activity against plant pathogens of metabolites from the endophytic fungus Cladosporium cladosporioides. J Agric Food Chem. 2013;61(19):4551-5.
19. Furtado NAJC, Said S, Ito IY, Bastos JK. The antimicrobial activity of Aspergillus fumigatus is enhanced by a pool of bacteria. Microbiol Res. 2002;157(3):207-11.