

Isolation and identification of antibiotic producing

microorganisms

Aarti Yadav¹, Sushma Dubey^{1*}

¹Department of Biotechnology, Kalinga University, Naya Raipur, Chhattisgarh-492101, India sushma.dubey@kalingauniversity.ac.in

Abstract

One of the most essential and widely used secondary metabolites produced by bacteria, fungus, and Streptomyces is the antibiotic. The soil is the best place to find microorganisms that produce antibiotics. Soil is a diverse supply of bacteria that produce antibiotics because it is complex, heterogeneous and home to a variety of microorganisms. The majority of antibiotics used today are produced in artificial media, isolated from soil microorganisms and extracted. With the use of an antibiotic produced by a bacterium, some human diseases caused by bacteria, fungus, and protozoa can be healed. The antifungal and antibacterial capabilities of fungi like *Penicillium* and *Cephalosporium* make them important sources of secondary metabolites and vital for the synthesis of industrial enzymes. Because they are more affordable and effective at stopping the growth of other organisms, the common soil bacteria known as bacillus species have been proven to be beneficial. The population of microorganisms is dependent on a number of characteristics, including the type of soil, temperature, water-holding capacity, oxygen pressure, pH, carbon supply, salt concentration, organic matter, aeration, moisture content, and others.

Keywords: Antibiotics; soil; micro-organism.

1. Introduction

Antibiotics are the medicines used to treat bacterial infections. They kill or prevent them from replicate or inhibit growth. In the form of secondary metabolites with antimicrobial properties, soil microbes create a variety of antibiotic(C.H. et al., 2020). Antibiotics are much more effectively obtained by isolating them from microbes than by chemically synthesis. Today, the antibiotics that used are isolated from soil microbes like fungi and bacteria. A certain human diseases caused by bacteria, fungi and protozoa can be cured with the help of antibiotic produced by microorganism.

These antibiotic is used in many aspects of human growth, including agriculture, human health, veterinary science, animal husbandry, and livestock maintenance.

Over 4000 antibiotics have already identified, and research is ongoing to find and use more of them. The soil is the best sources for antibiotic producing microorganism. A number of microorganisms live in soil, which is complex and heterogeneous in nature and a versatile source of microorganisms that produce antibiotics (Uwalaka et al., 2019). A teaspoon of soil includes between million to billion active microbes. The ability to produce more antibiotics allows some microorganisms to live longer than those who can produce a small quantity (Bavishi et al., 2017). Numerous factors including soil type, temperature, pH, salt concentration, carbon source, oxygen content, pressure, and others affect the microbial population in soil(Mannan et al., 2017). The most prevalent antibiotic discovered from soil are fungal and bacterial genus bacillus, streptomycin, penicillium and cephalosporium. Most antibiotic use today are isolated and extracted from soil microbes and are grown in artificial media(C.h et al., 2020);(Bhuyan&yadav et al., 2020).The common soil bacteria known as bacillus species have been found to be less expensive and more efficient at preventing the growth of other organisms because they are simple to isolate, cultivate, and keep(Kaur's & Pankaj et al., 2014). The main antibiotic producers of this genus are B. brevis (e.g., gramicidin, tyrothricin), B. cereus (e.g., cerexin, zwittermicin), B. circulans(e.g., circulin), B. laterosporus (e.g., laterosporin), B.licheniformis (e.g., bacitracin), B. polymyxa (e.g., polymyxin, colistin), B. pumilus (e.g., pumulin), B. subtilis (e.g., polymyxin, difficidin, subtilin, mycobacillin, bacitracin)(Marahiel et al., 1993; Berdy et al., 1974; D'Ave et al., 1997). For the purpose of discovering a novel antimicrobial producing, soil from various ecosystems and bioregions can be examine. Even though there are numerous antibiotics that are known to exist, research to find new antibiotics is still ongoing. As a result, the potential of numerous species to manufacture antibiotics, including Streptomyces, Bacillus, and Penicillium has been consistently researched (Sethi & Kumar et al., 2013).

Demand for a new antibiotic is increasing daily. Antibiotics with significant medicinal value have been found (Begum et al., 2017). Nowadays research is looking for microbes in an efforts to increase the number of antibiotics discoveries for the benefit. There are many different sources from which antibiotics can be discovered (Bavishi et al., 2017).

According to the International Center of Information on Antibiotics, 338 distinct types of fungi can produce antibiotics. The majority of antibiotics produced by fungi are used in chemotherapy especially fusidic acid. Fungal species including penicillium, and cephalosporium which have both anti fungal and antibacterial properties and are significant source of secondary metabolites and essential sources for industrial enzyme production (Rafiq et al., 2017).

There are many applications of antibiotic. Antibiotic like streptomycin and oxytetracycline are used to stop infection in the plant that produce vegetable fruits and crops. Antibiotics are also used in aqua culture, agriculture. Numerous antibiotic like erythromycin, sarafloxacin, oxytetracycline, premix have been used in aquatic animal farming. Multiple infections with bacteria have emerged as a result of modern aquaculture, driving up the need of antibiotics. In general, the use of these medications aims to treat illnesses, improve fish health, and to improve environmental quality. However, the use of such medications in commercial fish culture may put a greater stress on the microbial ecology and promote the development of natural resistance to antibiotics (Effend& Tanjung et al.,2020).

As we know different antibiotic have different mode of action. According to the mode of action might be undergoing each of following groups:

For wall synthesis- Penicillin, Cephalosporin, Bacitracin, Vancomycin

For Protein synthesis-Erythromycin, Neomycin, Streptomycin, Tetracycline, Vancomycin.

For Cell membrane- Polymyxin B, Amphotericin B, Colistin

For nucleic acid synthesis- Quinolones, Metronidazole and Rifampin(دادعا et al., n.d.)

2. What are Antibiotics

History

Dr.Alexander flaming first discover penicillin from a fungus in 1928 it is the first natural occurring antibiotic by chance when staphylococcus agar plate got contaminated by mold and the clear zone of bacterial inhibition itself(Hutchings et al.,2019) The development of antibacterial substances made by living organisms began with this finding. Streptomycin, a different antibiotic that has proven to be very effective against tuberculosis, was discovered in 1944 by microbiologist

Waksman from a kind of soil bacteria called Streptomyces griseus, notably tubercle bacilli. Burkholder discovered Chloromycetin, another antibiotic, in 1947. Apart from S. venezuelae, it was isolated. It has a powerful impact on various kinds of Gram positive and Gram negative pathogenic microbes (Makut& Owolewa et al.,2011).

An anti means against and bio means life (Sowjanya et al.,2020). An antibiotic is defined as "a substance produced by a microorganism (as a bacterium or a fungus) and in diluted solution having the capacity to inhibit the growth of or kill another microorganism (such as a disease germ)" in Webster's Third International Dictionary from 1981(Davies et al., 2006). They are the chemical substance secreted by the microbes which stops the development of other microbes and growth. Antibiotics can be consumed orally as liquid capsules. Antibiotics are used to treat a variety of infections in different ways. Antibiotics are the low molecular weight organic compounds and it's was realized that higher form of life like algae, animal and plant also have antimicrobial properties as secondary metabolites (Vishwakarma et al.,2017). Antibiotics produced by the soil microorganisms have very useful for curing the certain human diseases which are caused by the bacteria, fungi and protozoa (Sethi& Kumar et al.,2013).

3. Soil as a source of antibiotic producing microorganisms

As we know soil is the best source for microorganism which produces antibiotic as secondary metabolites and have low molecular weight organic compounds (Khalid and Rashid et al., 2016). Soils are the topmost layer of the earth crust. Each form of the soil have different properties and support for food production, obtaining raw materials for manufacturing. Soil have many components like minerals, organic materials, Soil organisms, water, air and also have trace elements. The dead and decay matters of the plant and animal are mostly composed of phosphate, carbon, nitrogen sources (Oluwatoyin et al.,2022). Soil plays important role in purifying freshwater and protecting ecosystems. There are different types of microorganisms found in the soil like bacteria Fungi, protozoa, algae and viruses (Sethi& Kumar et al.,2013).

Soil has a wide variety of microorganisms. Microorganisms are small organisms that can be found in food, water, soil, animal intestines, and other places. They can be classified as bacteria, fungi, alga, protozoa, and viruses (Oluwatoyin et al.,2022). The population of microorganisms are depends upon the various factors like soil type, temperature, water holding capacity, oxygen

pressure, pH, carbon source, salt concentration, organic matters, aeration, moisture content and other(Uwalaka et al., 2019; Abdulkadir &Waliyu et al., 2012).

4. Different antibiotics producing microorganisms

Bacteria

Bacteria is widespread microorganisms in the soil and are unicellular, prokaryotic bacteria without chlorophyll and nucleus. Plasmids are additional genetic components that some bacteria contain. These bacteria are might be dangerous when they infect positive processes like fermentation (used to make wine, for example) and breakdown. Bacteria are classified into five groups according to their basis shapes like spherical (cocci), rod (bacilli), spiral (spirillia), comma (vibrio) and spirochetes. They can exist single cells inpairs, chains or clusters(Gupta et al.,2017). Pseudomonas, Arthrobacter, Achromobacter, Clostridium, Enterobacter, Sarcina, Micrococcus, Cyptophaga, and Chondrococcus are a few of the bacterial genera that are most frequently found in soil(Oluwatoyin et al.,2022). Due to their ability to create resistant endospores and produce essential antibiotics like bacitracin and other similar compounds, Bacillus species, a filamentous species, are the most common soil bacterium(Gupta et al.,2017). There are numerous bacteria that have the ability to produce antibiotics. Some examples include bacillus species that produce antibiotics like bacitracin, pulumlin, and gramicidin that are active against gram positive bacteria like staphylococci, streptococci, corynebactor, and Streptomyces species as well as lactobacillus species that produce nisin that is active against gram negative bacteria (Rafiq et al., 2017).

Fungi

A filamentous mycelium-containing soil microorganism is referred to as a fungus. Antibiotics are made by fungi naturally. Fungi help in the initial decomposition of fresh organic compounds and are resistant to acidic soil conditions. The most common soil fungus include Alternaria, Aspergillus, Botrytis, Cladosporium, Cephalosporium, Haetomium, Mucor, Monilia, Penicillium, Fusarium, Rhizopus, Gliocladium, Trichoderma, Pythium, and others (Oluwatoyin et al.,2022). Worldwide, there was a huge variety of fungi. Fungi have an estimated 1.5 million species, of which between 70 and 100,000 have been found. (Kirk et al.,2008). The best source of antibiotics is fungi, therefore looking for antibiotics there has a lot of possibility. There are 338 different species of fungi that can produce antibiotics. Chemotherapy is where most of the antibiotics made

by fungus are used. Numerous fungal species, such as Penicillium, Aspergillus, Cladosporium, and yeasts, which were formerly thought to only be used as food, are now recognised as being the primary suppliers of enzymes and secondary metabolites for industry. A major supply of powerful new medicinal drugs comes from fungi. Penicillium and Cephalosporium, two species of fungi with antifungal and antibacterial capabilities, are important sources of secondary metabolites that are essential raw materials for the synthesis of industrial enzymes. (Rafiq et al., 2017).

Actinomycetes

Antibiotics' most well-known byproducts are actinomycetes. These microorganisms similar to fungus and bacteria in terms of their traits. (Gupta et al.,2017). They have conidia and hyphae like fungi and are unicellular. Due to characteristics, these microbes have been classified into a distinct category. Actinoplanes, Nocardia, Streptomyes, and Micromonospora are the four most common genera in soil. (Oluwatoyin et al.,2022). Actinomycetes are a particular kind of bacteria that, due to their elongated cells and ability to divide into filaments or hyphae, morphologically resemble fungus. They are gram-positive bacteria with high levels of cytosine (C) and guanine (G). (Gupta et al.,2017). Actinomycetes are substances that are beneficial for treatment. Actinomycetes naturally degrade substances like chitin or cellulose during the composting process. Thermopile and thermo tolerance are primarily responsible for the degradation of organic materials Some thermophilic and thermo tolerant Actinomycetes have been known to cause allergic respiratory symptoms. Currently used antibiotics include streptomycin, gentamycin, and rifamycin. Erythromycin, a product of Actinomycetes, is essential for both the pharmaceutical and agricultural industries. (Jeffery et al.,2006).

5. Conclusion

As well known, there is an increasing need for novel antibiotics. Soil from various ecosystems and bioregions can be examined in order to find a unique antimicrobial producing. Even though it is known that there are many antibiotics, research to discover new antibiotics is still taking place. A large number of fungi-produced antibiotics, particularly fusidic acid, are utilised in chemotherapy. Penicillium and Cephalosporium, two fungi with antifungal and antibacterial capabilities, are important sources of secondary metabolites and crucial raw materials for the synthesis of industrial enzymes. Modern aquaculture has given rise to numerous bacterial diseases,

increasing the demand for antibiotics. The usage of these drugs generally attempts to reduce illnesses improve fish health, and quality. However, the use of such medications in commercial fish culture may put a greater stress on the microbial ecology and promote the development of natural resistance to antibiotics. Even though it is known that there are many antibiotics, research to discover new antibiotics is still underway. As a result, various species, such as Streptomyces, Bacillus, and Penicillium, have been continuously studied for their ability to produce antibiotics.

References

- 1. Bavishi, A., Dalal, R., & Singh, S. (2017). SCREENING, ISOLATION AND IDENTIFICATION OF ANTIBIOTICS PRODUCING MICROORGANISMS FROM POTENTIAL SOIL SAMPLES OF WESTERN REGIONS OF MUMBAI. 6.
- 2. C.h, N. S., D, R. B. R., K, R., K, L. S., K, V. D., & Lakshmi, B. (2020). Isolation of Antibiotic Producing Microorganisms from Two Soil Samples of Phirangipuram, Guntur. World Journal of Current Medical and Pharmaceutical Research, 211–213.
- Mannan, S. (2017). Identification of Antibiotic Producing Bacteria from Soil Samples of Dhaka, Bangladesh. Journal of Microbiology & Experimentation, 4. https://doi.org/10.15406/jmen.2017.04.00134
- Uwalaka, E. I., Kassim, Z. J., Abdulmumin, A. R., & Hamzat,. (2019). Isolation and Identification of Antibiotic Producing A Bacteria from Soil Samples of Abattoir in Lapai, Niger State, Nigeria. Journal of Applied Life Sciences International, 1–8. https://doi.org/10.9734/jalsi/2019/v22i230123
- 5. Marahiel, M. A., Nakano, M. M. and Zabar, P., 1993. Regulation of Peptide
- 6. Antibiotic Production in Bacillus. Mol Micro 7, pp. 631-636.
- 7. Berdy, J., 1974. Recent Developments of Antibiotic Research and Classification of
- 8. Antibiotics According to Chemical Structure. Adv. Appl. Microbiol, 18: pp. 309-406.
- D'Ave, B.., 1997. In: Textbook RSA, G. and Stern, G.A. (1997). Peptide Antibiotics: Vancomycin, Bacitracin, and of Ocular Pharmacology (eds. T.J. Zimmerman, K.S. Kooner, M. Sharir and R.D. Fechtner), Lippincott-Raven, Philadelphia. pp. 549 -552
- 10. Hopwood, D. A., & Merrick, M. J. (1977). Genetics of antibiotic production. *Bacteriological reviews*, 41(3), 595-635.

- 11. Isolation and identification of Bacillus species from soil and evaluation of their antibacterial properties. *Avicenna Journal of Clinical Microbiology and Infection*, *2*(1).Khalid, Z. Z., Rashid, F., Ashraf, A., Iqbal, M. N., & Hussain, F. (2016). Isolation and screening of antibiotic producing bacteria from soil in Lahore city. *PSM Microbiology*, *1*(1), 1-4.
- 12. Vishwakarma, R. (2017). Isolation, identification, purification, and characterization of antibiotic-producing bacteria from different soil samples. *International Journal of Advance Research, Ideas and Innovations in Technology*, *3*(5), 196-204.
- 13. Begum, K., Mannan, J. S., Rahman, M. M., Mitchel-Antoine, A., Opoku, R., Burgos, N., ... & Nur-E-Kamal, A. (2017). Identification of antibiotic producing bacteria from soil samples of Dhaka. *J. Microbiol. Exp*, 4, 00134.
- 14. Davies, J. (2006). Are antibiotics naturally antibiotics? *Journal of Industrial Microbiology* and Biotechnology, 33(7), 496-499.
- 15. OLUWATOYIN, P. (2022). ISOLATION AND CHARACTERASATION OF ANTIMICROBIAL PRODUCING BACTERIA ASSOCIATED WITH THE SOIL.
- 16. Hutchings, M. I., Truman, A. W., & Wilkinson, B. (2019). Antibiotics: past, present and future. *Current opinion in microbiology*, *51*, 72-80.
- 17. Kaur, S., Kaur, J., & Pankaj, P. P. (2014). Isolation and characterization of antibiotic producing microorganisms from soil samples of certain area of Punjab region of India. *Int J Pharm Clin Res*, 6(4), 312-315.
- 18. Bhuyan, A. P., Yadav, R. N. S., & Samanta, R. (2017). ISOLATION AND CHARACTERIZATION OF ANTIBIOTIC PRODUCING BACTERIA FROM SOIL SAMPLES OF DIBRUGARH DISTRICT, ASSAM. *International Journal of Pharmaceutical, Chemical & Biological Sciences*, 7(3).
- 19. Sethi, S., Kumar, R., & Gupta, S. (2013). Antibiotic production by microbes isolated from soil. *International Journal of Pharmaceutical Sciences and Research*, 4(8), 2967.
- 20. Abdulkadir, M., & Waliyu, S. (2012). Screening and isolation of the soil bacteria for ability to produce antibiotics. *European Journal of applied sciences*, *4*(5), 211-215.
- 21. Effendi, I., Tanjung, A., & Sari, D. M. (2020, October). Isolation of antibiotic-producing bacteria from extreme microhabitates in mangrove ecosystem. In *Journal of Physics: Conference Series* (Vol. 1655, No. 1, p. 012019). IOP Publishing.

- 22. Makut, M., & Owolewa, O. (2011). Antibiotic-producing fungi present in the soil environment of Keffi metropolis, Nasarawa state, Nigeria. *eubacteria*, 10(18), 19.
- 23. Dutta, A. C., & Dutta, T. C. (1964). *Botany for degree students* (No. 581 DUT). London: Oxford university press.
- 24. Hopwood, D. A., & Merrick, M. J. (1977). Genetics of antibiotic production. *Bacteriological reviews*, 41(3), 595-635.
- 25. Emmanuel, A. J., & Igoche, O. P. (2022). Isolation and Characterization of Antibiotic Producing Fungi from Soil. *Microbiology Research Journal International*, 32(9), 28-40.
- 26. Kirk, P. M., Cannon, P. F., Minter, D. W., & Stalpers, J. A. (2008). Dictionary of the fungi. CAB International. *UK.*[Google Scholar].
- 27. Jeffrey, L. S. H. (2008). Isolation, characterization and identification of actinomycetes from agriculture soils at Semongok, Sarawak. *African Journal of Biotechnology*, 7(20).