Characterization of antimicrobial agent from bacterial isolates of hospital waste sites against *Pseudomonas aeruginosa*

SUMMARY OF THESIS

SUBMITTED TO

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

(A CENTRAL UNIVERSITY)

LUCKNOW

FOR THE DEGREE OF

Doctor of Philosophy

IN

ENVIRONMENTAL MICROBIOLOGY

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2024

Summary

The rate of discovery of new antibiotic is slower than the emergence of antibiotic-resistant strains in the environment. This global problem is more acute in developing countries. Therefore, it is necessary to develop some alternative approaches to combat infections caused by pathogenic microorganisms and resistant strains. Natural antimicrobial peptides (NAMPs) are potent antimicrobial peptides that are isolated from different sources like plants, animals, humans, bacteria, and fungi. These antimicrobial peptides may have a ribosomal or non-ribosomal origin. Natural antimicrobial peptides have diverse functions in agriculture, pharmaceutical and food industries(Kumar et al., 2020). NAMPs have been used as food preservatives against food-borne pathogens thereby increasing the shelf-life of food items. NAMPs are useful in the treatment of wounds, ulcers, skin and soft tissue infections caused by microorganisms. Different types of NAMPs are universal in nature and show broad-spectrum antimicrobial activities. NAMPs exhibit great potency against multidrug-resistant bacteria like methicillin-resistant *Staphylococcus aureus* (MRSA)They have unique characteristics of targeting multiple pathogenic strains and prevent the emergence of natural resistance (Kumar et al., 2020)

The hospitals are a high-risk workplace. In addition to the risks specific to any organizational structure, there are repalce with risk linked to its activities:such as pathogens, use of sensitive technologies or devices, dealing with stress, suffering, and death (WHO 2020). Healthcare activities generate waste with varying degrees of risk for the patient, staff, and the environment.(Chartier Y (Ed)., 2014) The production of healthcare wastes (HCWs) is increased during special situations such as epidemics.(de Aguiar Hugo et al., 2021). Healthcare facilities have an important economic and social role. During the course of the healthcare delivery process, HCWs are generated, a large part of which is assimilated to household waste (packaging, kitchen waste, green waste, etc.) and another category which may have a risk to health and the environment given its nature and typology. This category includes waste at risk of infection such as stinging, sharp waste (needles, blade, scalpel, etc.), and waste at chemical risk. (Chandrappa R et al.2024)Among the health impacts of the mismanagement of HCWs, ~~we can cite,~~ at the international level, 16,000 hepatitis C (HCV) infections, 66,000 hepatitis B (HBV) infections, and approximately 1,000 infections with virus of human

immunodeficiency (HIV), leading to approximately 1,100 deaths and significant disability.

Pseudomonas aeruginosa is an opportunistic pathogen of human and animals which may be spread by water, food and air. (Mena, K. D., & Gerba, C. P. 2009). The major source of *P. aeruginosa* in water appears to be fecal wastes of human and animals .The significance of *P. aeruginosa* as an indicator of potential health hazards associated with water must be judged on the basis of its own role as a waterborne pathogen. The role of *P. aeruginosa* as a waterborne pathogen, its sources, and its behavior in aquatic environments are reviewed by Mena, K. D., & Gerba, C. P. 2009. Its isolation from drinking water, farm water supplies, swimming pool water and surface recreational waters should be regarded with concern. However, while authorities have recommended limitations on *P. aeruginosa* in waters used for various purposes, few epidemiologic studies have been undertaken to understand the base level, and few standards have been established.

this study, we have explored the potential of hospital waste soil site soil samples for the Identification of potential bacteria which may produce broad spectrum antimicrobial agent against several Gram-positive and Gram-negative pathogens. We have collected the soil samples from the hospital waste site soil From district hospitals of Uttar Pradesh, India, and subjected them to exhaustive primary, secondary, and tertiary screening for isolation of potential isolates that showed antimicrobial activity against *Escherichia coli* MTCC 1304, *Bacillus subtilis* ATCC 6633, *Salmonella typhi* MTCC 581, *Shigella flexneri* MTCC 9543, *Pseudomonas aeruginosa* ATCC 2785 and *Staphylococcus aureus* ATCC 23925.

This thesis is compiled into NINE CHAPTERS. CHAPTER FIRST is a general introduction related to the research topic. This chapter has explained the detailed background information Related to the antimicrobial agents.Secondary metabolites are organic compounds that are not directly involved in the normal growth, development and reproduction of organisms. Secondary metabolites or Antimicrobial metabolites are produced By bacteria to inhibit other organism's Growth for same ecological niche. Secondary metabolites are produced after active growth of the organism and are structurally diversified. The distribution of secondary metabolites is also unique and

some metabolites are found in a range of related microorganisms, while others are only found in Specific species.

In CHAPTER SECOND, information related to the entire history of antimicrobial agents, classification and diversity of natural antimicrobial peptides and ~~explored~~ sources of antimicrobial agents. Many soil-inhabiting bacteria are known to produce secondary metabolites that can suppress microorganisms competing for the same resources (Garbeva et al., 2011). Microbial population play's a prominent role In biotechnology and pharmaceutical industries as it offers countless new genes and biochemical pathways to probe for enzymes, antibiotics and other useful molecules. General details of *P. aeruginosa* regarding Its pathogenesis. *P. aeruginosa* is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes. *P. aeruginosa* is able to selectively inhibit various antibiotics from penetrating its outer membrane and has high resistance to several antibiotics. According to the World Health Organization *P. aeruginosa* poses one of the greatest threat to humans in terms of antibiotic resistance. The development of extra-chromosomal genetic components containing genes that impart resistance to certain antibiotics is one mechanism through which antimicrobial resistance is genetically imposed. However, investigations of microbial sources for secondary metabolites as potent antimicrobial agent Is highly needed to combat multi drug resistant strains.microorganisms have enormous potential to produce a variety of antimicrobial agents such as bacteriocins (nisin) a bacterial metabolism-derived compound.CHAPTER THREE has written all four objectives related to my thesis topic. CHAPTER FOUR mainly focused on materials and methods used in sample collection, isolation, and screening of antimicrobial agent-producing bacteria by well diffusion method. Characterization of most potential isolates BU-1 and BU-2 through 16S rRNA method. Growth kinetics was done in 1 to 10 days and active compounds were extracted by an ethyl acetate solvent system. Active compounds have been separated by HPLC, and identified by FTIR and ESI-MS technique. Separated compounds MIC was calculated by Microtitre plate method and ~~all~~ compound's anti-biofilm efficacy was tested against *P. aeruginosa*. The bacterial diversity of the BTC2 sample was also done by metagenomics approach in

which DNA of the soil sample was extracted by using Xploregen DNA extraction Kit (Xploregen Discoveries, India) according to the manufacturer's instructions and the functional metabolic pathway identified by Microbiomeanalyst.

The CHAPTER FIVE has described the results. The findings of the study revealed that screened most potential isolates BU-1 and BU-2 by molecular methods identified as *bacillus licheniformis* RG1002 and (OM438151) *bacillus amyloliquefaciens* strain RG1001 (0M438150), FTIR and ESI-MS analysis suggested the bioactive compound belongs to Lichenysin and Fengycin A, with molecular weight 1035.2 Da and 1463.7 Da. We further calculated the MIC of lichenysin, (25µg/ml) Fengycin A (6.26µg/ml) against *P. aeruginosa*. Further, the metagenomics of the BTC 2 soil sample revealed that Hospital waste site soil showed a higher abundance of phylum firmicutes (58.37%) and Proteobacteria (22.97%) present in BTC 2.

The SIXTH CHAPTER has discussion and conclusion of my Work. In this chapter, we have discussed: Two potent isolates BU 1 and BU 2 in which all isolates were Gram positive.The quantitative analysis of biofilm inhibition in the presence of Lichenysin at MIC 25 µg/mL and fengycin A at MIC 6.25µg/mL suggests that *P.aeruginosa* was not able to form biofilm as compared to positive control.

All the extracted bioactive compounds had good anti-biofilm and bactericidal activity that might be used to prevent bacterial biofilm and bacterial infections in hospitals. These compounds would be useful for industries and research as future antibiotics that would replace synthetic antibiotics. This assumes that it follows a special mode of action against pathogenic bacteria where its target will not be interfering with the eukaryotic system. Bacterial diversity analysis also uncovers a new perspective on land use management for sustainable development.

CHAPTER SEVEN has summarized the entire thesis work, results, and findings with scientific output systematically. CHAPTER EIGHT contains all the references cited in the thesis chapter related to the topic. I have cited recent references in each chapter. The cited references are related to natural antimicrobial agents against *P. aeruginosa* from the hospital waste site soil. There are 420 references cited throughout the entire thesis.

The last NINTH CHAPTER has compiled and listed all the scientific output. I have published 5 research papers, three review papers, and one chapter in reputed Journals and international conferences, I have received certificates of participation and awards for oral presentations.