Identification of MDR resistance bacterial isolates from industrial wastewater

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Abstract

One of the major environmental issues today industrial wastewater sewage and wastewater microorganisms has the ability to survive in extreme conditions and during this adaption process; they develop distinctive properties which could be of immense significance for mankind. Extremophiles come in this category and are further classified based on the environmental challenge they are facing, which constitute one of the major qualities to survive in presence of the pathogenic microorganisms. The purpose of this study is to investigate the performance of isolated bacteria with various test pathogens to get anti-microbial activity and other enzymes produced by the microbial flora in its attempt to enhance the process of microbial resistivity, three bacterial isolates showed the maximum antimicrobial activity against pathogenic bacteria, the above study showed that the isolated bacteria have MDR property and this will be further investigated with another pathogenic microorganism.

Keywords: wastewater, MDR resistance, anti-bacterial activity and pathogenic bacteria.

Introduction

Recent development of industry or more industrialization in India, main cause of high concentrations of toxic wastes in the environment is of great concern. Various industrial activities such as textiles, tannery, agro-food production, chemical manufacturing and oil/gas production, mine and thermal power, and pharmaceuticals industry generate waste water with high concentration of hazardous waste and such wastewater sludge basically consists of hydrocarbons, aliphatic and aromatic, oil and grease, antibiotic, heavy metals and pathogenic microbes (Includes bacteria, fungus and viruses). (Saranya, Sekaran & Ramani, 2014; Pazda, et al 2019) These wastes are toxic to all forms of life, including humans and exhibits mutagenic, teratogenic and carcinogenic effects on biological systems. (Kumara & Pal, 2018)

Wastewater and wastewater treatment plant is hot spot for extremophilic microorganisms and antibiotic resistant microorganisms. (Karkman, et al. 2018; Ndlovu et al.2018). It involves utilization of microbial strains to metabolize the toxic pollutants and convert them into useful products by help of enzymatic activity, numerous studies conducted in the past few decades have identified for isolation of antibiotic and multidrug resistant microorganism from wastewater (Wang et al,2020; L. Jałowiecki et al., 2017). In the current world, necessity of new antibiotics is increasing with time due to the development of counter drug resistance by the pathogenic organism. (Pazda et al.,2019) Origin of new forms of pathogenic organisms from existing organisms is also leads to the frustration in the scientific communities (Battu et al.,2009; Federle et al.,2003). Process of developing a drug and its validation is somewhat tedious and ample amount of time has been utilized in this process. (Wang et al., 2020; Sabri et al., 2018).
In these situations natural products obtained from the environment with an effective antimicrobial activity can be used as a replacement of these drugs. As the discovery of novel chemical classes have been in decline for the past two decades, the need to exploit new resources in search for effective chemicals with novel mechanism of actions is imperative.

In these situations wastewater microbes could be explored for novel and rich sources of biologically active compounds. Till date ~18000 compounds have been screened and discovery of these compounds has been observed due to the interest of various pharmaceutical industries.

Study on microorganism existing in industrial sewage wastewater has limitation because of the extremely complex and presence of huge diversity of life forms. During the time of their survival in this environment they live in close association other organisms and toxic sludge, which lack obvious structural defence mechanisms, and thus rely on chemical defence by production of bioactive secondary metabolites, either by themselves or by associated micro flora, to survive in their extreme habitat (Jensen et al., 1994). Currently, many investigators targeted on wastewater sources for the identification of unusual metabolites. (Lam et al., 2006). Moreover, from wastewater source, a huge number of biologically active compound with various action such as antibacterial, antifungal, anti-diabetic, anti-inflammatory, antiprotozoal and antiviral activities have been reported so far (Mayer et al., 2013).

Several sources have been exploited to find out the microorganisms present in the environment with an ability of producing compound with prime importance. Wastewater environment is such a resource containing the diverse population of microorganism and yet to be tapped for the potential it can offer.

In continuation to our previous studies, in this research work we tried to extract the secondary metabolites compounds produced by extremophiles and their identification for the development of future antimicrobial drugs.

1. Materials and Methods

1.1 Collection sediment samples:

Wastewater sediment samples were collected from Tamil Nadu (Neyveli Lignite Corporation Limited) (11.60°N 79.48°E.) and Rajasthan (bagru dye industry) (26.82°N 75.55°E) India, at 10 cm depth. Samples were collected in a sterile zip bags and stored at 4ºC. The wastewater sediment samples were stored under sterile conditions for preventing the bacterial cross contamination until use.

1.2 Isolation and Identification of microbial Species:

In the present study the micro-organisms have been isolated from wastewater sediments and it appears in semi-solid form. The zobell marine agar was use for series dilution for neyveli lignite mine sample and nutrient agar medium was used for bagru dye sample and then both was sterilized in an autoclave at 15 lbs Pressure (1210C) for 15 minutes.

Each dilution was plated in sterilized Petri plates and 15 ml sterilized media was poured. The content of the plates mixed thoroughly and the media was then allowed to solidify for isolation of marine bacteria from each water sample, 1.0 ul of the sample was mixed with 9.0 ul of distilled water and serial dilution was performed up to 10-5. From this about 100ul of the aliquots was spread on the surface of the Media; triplicates were maintained for each dilution. The mixed consortia were isolated on the basis of antimicrobial potential against test pathogen and incubated at 25oC for 3-4 days. Different Colonies developed after incubation were pick up and purified by repeated sub culture, were preserved on Luria agar and finally, bacterial colonies with distinct characteristics such as pigmentation, size, opacity, elevation, margin and surface appearance (Yeon et al., 2005) were chosen for further characterization.

1.3 Biochemical Test

Bacterial colonies isolated from the nutrient agar with metal supplemements were morphologically characterized through their cultural characteristics, Gram staining and other biochemical characterization. Pure cultures obtained from the collected samples and isolated strain was identified by examining the morphological and biochemical characteristics following the Bergey’s manual of systematic bacteriology.
1.4 Optimization of culture conditions for pH and Time

To optimize culture conditions, various parameters like pH, salt, and incubation period were carried out on the ZMB and LB medium. Optimum incubation period was calculated for the isolates by incubating the culture at 25 °C at 150 rpm by keeping other parameters constant to attain the maximum of biomass. During this incubation period, sample was withdrawn at regular intervals and after centrifugation (8000 rpm for 20 min, 4 °C), antimicrobial activity of cell-free supernatant (CFS) was measured.

Once the culture conditions optimized, large scale pilot experiment was set up for metabolites extraction where overnight grown culture was used as a primary inoculum for further inoculating a large scale volume of media. Optimizing the environmental parameters can significantly affect microbial secondary metabolites rates of production. Growth conditions could be optimized by varying the addition of nutrients, adjusting soil moisture content, liming soil to adjust pH, or the addition of bulking agents to provide a primer carbon source and provide aeration.

1.5 Test bacterial strains

Two clinical Pathogen one is Gram-positive bacteria: Staphylococcus aureus, and another one is Gram-negative bacteria Pseudomonas aeruginosa were procured from the Medical college of Jaipur, (Rajasthan) India. All bacterial strains kept as stock culture in trypticase soy broth (tsb medium) containing 20%(v/v) glycerol at -70°C.

1.6 Bioassay of antimicrobial activity from wastewater sediments bacteria:

In preliminary screening, determination of the antimicrobial activity of around 124 bacterial strains isolated and screened for antimicrobial activity using dual culture assay. Of these, 4 isolates exerted an inhibitory effect against test organisms (Staphylococcus aureus and Pseudomonas aeruginosa). Further screening was performed by zone of inhibition method against the standard test organisms. Active microbes were cultured for the screening of antibiotic substances and analysis is further established to be detected in a various organic solvents of the crude extract; the secondary metabolites and partial purification of each compound obtained from secondary metabolites were demonstrated for antimicrobial activity against pathogenic bacteria

Antibiotic assay of isolated bacteria

In this screening isolated bacteria was check against antibiotic along with test pathogens via well-cut method, among four isolates, 3 of them showed antimicrobial activity against both the test pathogens and further it was test against antibiotic in two concentration (ug/ml), the first concentration was less and 2nd concentration was little high. NL-7 has high efficiency against antibiotic rest of the isolates showed less effective.

2. Result

Total 124 strains isolated from Neyveli mine wastewater and bagru dye wastewater was showed antimicrobial activity. The antimicrobial activity of wastewater bacteria of sediments had been reported previously, Out of these 4 bacteria NL-4, NL-5, NL-7 and BD-7 showed antibacterial activity to Staphylococcus aureus and Pseudomonas aeruginosa. All 4 isolated strains showed activity against both the human pathogen and is selected for further investigation. Wastewater bacteria showing antibacterial activities have been described for more than 50 years.
From this study we find out that antibiotic producing microorganisms are present in waste water sediments, regions of Tamilnadu and Rajasthan. Out of these 124 isolates, four produced antibacterial activity against the tested organisms. NL-7 is more potential isolates among rest of the isolates.

3. Discussion and Conclusion

Industrial wastewater bacteria have been recognized as important and untapped resource for antibiotic and novel bioactive compounds. Development of environmental biotechnology is expected to produce novel compounds that may contribute significantly towards drug development over the next decade. In this study, we isolated several bacteria for extraction of some possible types of antimicrobial. This work emphasizes the preliminary studies of antagonistic activity of bacteria still the work as to be optimized to be detect the secondary metabolites and partial purification of each compound obtained from secondary metabolites were demonstrated for antimicrobial activity against pathogenic bacteria.

Among the 124 strains isolated from various wastewater soil sediments, out of this 4 strains showed strong antagonistic activity against bacterial pathogen. Furthermore, the specific compound is responsible for antimicrobial activity will be analyzed by advance spectroscopic analytical technology. Then the mechanism of action of purified compounds will be further investigated for MDR antibiotic and potential isolates will be check for other pathogenic microorganism.

A. Structure

1. Figure: Serial dilution and Spread Plate Results
2. Figure: Four strains isolated from wastewater soil

3. Figure: Gram staining for selected samples

a) *Pseudomonas aeruginosa*
b) *Staphylococcus aureus*

![Image of Staphylococcus aureus strains](image)

4. Figure:- Isolated strain showing zone of inhibition against test pathogens

![Image of inhibition zones](image)

a) *Pseudomonas aeruginosa*  
b) *Staphylococcus aureus*

5. Figure :- Antibiotic resistivity test by well-cut method

### B. Tables

1. Table:- Basic description of Sample

| Place of Collection | Sample | Sampling site Code | Total No. of Strains Isolated | No. Of Isolates Shows zone of inhibition |
|---------------------|--------|--------------------|-------------------------------|---------------------------------------|
| Neyveli lignite     | NL     | 74                 | 3                             |
| Bagru dye           | BD     | 50                 | 1                             |

2. Table: - Isolates showing zone of inhibition against test pathogens

| Isolate Code | Zone of inhibition(mm) |
|--------------|-------------------------|


Table: Morphology of isolated bacteria

| Isolated bacteria | Colour/Appearance | Gram Staining  | Shape     |
|-------------------|------------------|----------------|-----------|
| NL-4              | White, Slimy     | Negative       | Rod       |
| NL-5              | White, Dry       | Positive       | Rod       |
| NL-7              | White            | Negative       | Rod       |
| BD-7              | White            | Positive       | Cocci     |

B.4 Antibiotic resistivity test showed zone of inhibition

| Isolate Code | Zone of inhibition(mm) | Staphylococcus aureus | Pseudomonas aeruginosa |
|--------------|-------------------------|-----------------------|------------------------|
|              |                         | Less concentration    | High concentration    |
|              |                         | Antibiotic (ug/ul)    | antibiotic (mg/ul)    |
| NL-4         | 8mm                     | Nil                   | 7mm                    |
| NL-5         | 8mm                     | Nil                   | Nil                    |
| NL-7         | 8mm                     | 6mm                   | 14mm                   |
| Control      | Nil                     | Nil                   | Nil                    |
|             |                         | High concentration    |                        |
|             |                         | Antibiotic (mg/ul)    |                        |
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