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ORIGINAL ARTICLE

Isolation, Characterization and Identification of Bacteria from Industrial Waste Soil and Antibacterial Profile of Bacillus

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ABSTRACT

Soil is a diverse environment in which different populations of microorganisms exist in different proportions. Soil facilitates the growth of many types of organisms as it contains a variety of nutrients that can be used as carbon source by microorganisms. Soil has the greatest capacity for accepting and decomposing pollutants of different kinds. Fifteen (15) different soil samples from industries of Karachi –Pakistan were collected and screened for their antibacterial activity. A standard procedure was employed for the isolation, identification and characterization of bacteria. Out of these samples, 7 different isolates of *Bacillus*, 4 different isolates of Staphylococcus, 2 different isolates of *Pseudomonas*, 1 isolates of *Micrococcus* and Serratia were isolated. Antibacterial activity was carried out using compound isolated from *Bacillus*. The compound was then isolated by centrifugation and assayed for its activity. The inhibitory activity of the Bacitracin was screened against *E.coli*, *Pseudomonas aeruginosa* and *S.aureus*. The research provides a step forward towards industrial development.

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Introduction

Soils provide an excellent cultural media for the growth of many types of microorganisms (1). Bacteria being the predominant group found in soil. Bacterial population differs with locality. Industrial waste pollutants cause important alterations in vegetation, soil, water, and the atmosphere and affect people (2). Some microorganism from soil waste, contaminated food stuff like *Bacillus* spores as a result cause food poisoning (3). *Bacillus* found in bakery and bakery equipment. Staphylococcus species are found in a broad range of commercial foods (4). Textile goods provide an excellent environment for microorganisms to grow. They produce fiber damage; unpleasant odors and a slick, slimy feel (5).

Soil bacteria have the ability to degrade industrial waste pollutants like, dyes (AZO) are harmful, poorly bio degradable, and there are few known micro organisms that reductively cleave AZO bonds under aerobic conditions (6). Some plastic products can be toxic. It interferes with hormone functions and suspected human carcinogens (7). Microorganisms destroy the contaminants (8).Microbial mineral precipitation technologies have already been used for sand consolidation and improvement strength of bricks (9).There are three general area of industrial application of the bacteria: enzymes, antibiotics and insecticides (10).

Bacitracin ($C_{66}H_{103}N_{17}O_{16}S$) produced by *Bacillus*, composed of polypeptides made up of 12 amino acids. Bacteriocin has antibacterial activity on gram positive bacteria but low activity against gram-negative bacteria. It functions as an inhibitor of cell wall biosynthesis. Microbes are able to produce enzymes that can destroy antibiotics (11)

The objectives of this study are (1) finding bacterial isolates from industrial waste soil (2) production of antibiotic from *Bacillus* and measuring its inhibitory effects against the growth of ***E.coli*, *Pseudomonas aeruginosa*** **and S. aureus.**

Materials and Method

Isolation, Identification and Characterization of Bacteria: Weighed 1gm of soil sample and added into 10ml saline under sterile conditions and perform serial dilution (10^{-1}, 10^{-2}&10^{-3}).0.5 ml of sample is collected from the last dilution test tube and plated on Nutrient agar using a sterile wire loop. The plates are inverted and incubated over night at 37^{0}C. Next day observe colonies, and performed gram staining, andperform biochemical test for further identification of bacteria.

Antibiotic Production: Sterilized broth is inoculated with test culture under aseptic conditions and is transferred to shaking water bath for 48 hoursat 120rpm. After 2 days, pelleted out cells at 6000 rpm for 10 mins and the supernatant is collected and stored at 4^{o}C. The Kirby Buer Method was performed using supernatant. The plates were incubated overnight without inverting at optimum temperature of 37^{o}C for 24 hours. Next day observed zone of inhibition.

Results

Out of 15 different industrial soil samples, from 7 samples *Bacillus* was isolated, from 4 samples ***Staphylococcus aureus***, from 2 samples ***Pseudomonas*** ,from 1 sample ***Micrococcus*** and from 1 sample ***Serratia*** was isolated. This result shows bacterial population is different with respect to locality. No Inhibition zones for ***S. aureus***, ***E.coli*** and ***Pseudomonas*** were observed around the region surrounding the wells that contain crude extract.

Table I: Antibacterial Activity of Bacitracin

S. No	Test Organism	Zone of Inhibition
1	*Pseudomonas aeruginosa*	No zone
2	*Staphylococcus aureus*	No zone
3	*E.coli*	No zone

Discussion

Contaminated soils have high bacterial profile as compare to non-contaminated soils (14). Bacteria are predominant in different industries (12). Some studies shows that bacterias like Escherchia, Shigella, Xanthmonas, Acetobacter, Citrobacter, Enterobacter, Moraxella and Methylococcus are most dominant genera isolated from industrial soil. However, less diversity of Gram-positive bacteria were recovered from industrial sites and represented by *Bacillus*, *Micrococcus*, and Staphylococcus. In our study the bacterial isolates identified were mostly represented by Gram-positive bacteria and less in no. of gram negative bacteria.

The pollution of the environment with heavy metals of industrial region has led to the appearance of heavy metal resistance microorganisms in the soil and water. Our data indicated that heavy metals resistance has been shown to be correlated with antibiotic resistance like *Pseudomonas* gives resistivity against antibiotic (Bacitracin).

Bacillus from the soil samples of different Industrial areas of Karachi were screened for their potential as a source of antibiotics (Bacitracin). Maximum Bacitracin production was obtained when 20 hours (11) Antibiotic production in this study revealed that isolates of *Bacillus* species produced very little inhibitory effect as noticed on *Pseudomonas aeruginosa*, and E. coli. However study previously conducted by Faruk Adamu et al., the inhibitory effect of *Bacillus* was more on Klebsiella species (83%), Streptococcus pyogenes (75%), Salmonella typhi (66.7%), Staphylococcus aureus (41.7%), E. coli (25%) and P. *aeruginosa* (16.7%) (13).

In present study the test microorganisms included *Pseudomonas*, *E.coli* and Staphylococcus aureus. The antibiotics were found ineffective against these bacteria. Test organisms have shown resistivity against Bacitracin in previous studies. Reason of their resistance might be the fact that, all of these are gram-negative bacteria that secrete exopolysaccharides acquired resistance to the antibiotic Bacitracin (14).

Conclusion

This research is a preliminary step towards the industrial application of the useful bacterial strains for the manufacturing of antibiotics which also provide the control

against gram positive bacteria. Strain was cultured from soils so its isolation, culturing and industrial development aspects will be very easy to control and optimize as well.

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