ISOLATION AND SCREENING OF SOIL BACTERIA FOR POTENTIAL ANTIMICROBIAL ACTIVITY

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Abstract

Antimicrobial agents are the most promising field worldwide with the need of continuous search of new ones. Present study was to investigate and explore our indigenous soil microflora for antimicrobial production. Among 22 isolated organisms, 14 were identified as *Bacillus* sp., 5 were *Pseudomonas* sp. and 3 were *Escherichia coli*. One of the identified organisms as *Escherichia coli* was found to have activity against clinical isolates *i.e. Cornybacterium pseudodiphtherieae, Pseudomonas vulgaris and Staphylococcus aureus* and another identified *Bacillus* sp. showed high activity against *Escherichia coli*.

Introduction

Natural products from microorganisms have been the most successful source that has found many applications in the fields of medicine, pharmacy and agriculture. Most of the antibiotics in current use for the treatment of various infectious diseases are microbial products (Tawiah *et al.*, 2012). Studies on soil bacteria and fungi have shown that these microorganisms are potentially rich source of unique bioactive substances (Fenical, 1993).

Since the discovery of penicillin and other antimicrobial agents by Alexander Fleming in 1928 (Fleming, 1929) to date, there has been continue search for more effective antibiotics that can stand the emerging menace of drug resistance among microorganisms worldwide (Kuta, 2008; Guy *et al*, 2008; Song, 2008). In Pakistan, resistance to antibiotics has been reported by many researchers which resulted in treatment failures and increased health care costs (Hassan *et al*, 2011). The increase in antibiotics resistance is prevailing due to inappropriate use of antibiotics by general health practitioners and leads to the effectiveness of front line antibiotics worldwide (Kuta *et al*, 2008; Blomberg, 2008; Byarugaba, 2004). This situation has become an alarming condition to drug manufacturers and public health practitioners. Therefore, this study is an attempt to investigate indigenous soil resources with potential of antimicrobial production that could be used to produce new product with better efficacies.

Material and Methods

Isolation of Bacterial Strains: Soil sample was collected from Garden area of Department of Chemistry, University of Karachi Karachi, Pakistan .Soil was collected in a sterile zipper bag and processed aseptically. 0.5g soil was taken in 4.5ml sterile saline tubes mixed thoroughly. Sample was then serially diluted and plated on nutrient agar (Oxoid Cambridge, UK). Plates were then incubated at 37^{o}C for 24hr.

Culture Identification and Preservation: Cultures were purified by streak plate method and isolated strains were identified by conventional techniques i.e. Gram and spore staining and biochemical characterization as described by Bergey's Manual. Cultures were maintained on agar slants and preserved in 40% glycerol at -70^{o}C.

Test microorganism: Test microorganisms kindly provided by local diagnostic laboratory were used in this study: *Escherichia coli, Staphylococcus aureus, Pseudomonas vulgaris, Cornybacterium pseudodiphtherieae, Proteus sp.*

Screening of cultures for antimicrobial activity: The isolated strains were then screened for antimicrobial activity against standard clinical isolates; *Escherichia coli, Staphylococcus aureus, Pseudomonas vulgaris, Cornybacterium pseudodiphtherieae, Proteus sp.* The screening of potential strains was done by cross streak method as described by Nanjwade *et al.* 2010

Results and Discussion

A total of 22 culture strains were collected from soil sample collected from Garden area of Department of Chemistry, University of Karachi, Karachi, Pakistan. The cultures were isolated and further characterized on the

basis of colonial morphology, Gram' staining, spore staining and biochemical tests. Among the isolated strains, three were *Escherichia coli*, five of them were *Pseudomonas* sp., fourteen of the isolates were *Bacillus* sp. The cultures were cross streaked against test organisms for antimicrobial activity. Out of the total isolates two cultures were positive for having antimicrobial activity (Table 1).

Table. 1 Cultures showing antimicrobial activity against test organisms.

Test Organisms	Culture # 8 (*Escherichia coli*)	Culture # 21 (*Bacillus sp.*)
Escherichia coli	-	+++
Pseudomonas vulgaris	+	-
Staphylococcus aureus	+	-
C. pseudodiphtheriae	+	-
Proteus sp.	_	-

One of the culture belonged to *Bacillus* sp. showed high antimicrobial activity against *Escherichia coli* (Fig. 1) while other culture which was identified as *E. coli* from our collected isolates shown antimicrobial activity against *Cornybacterium pseudodiphtherieae, Pseudomonas vulgaris and Staphylococcus aureus.* (Fig. 2).

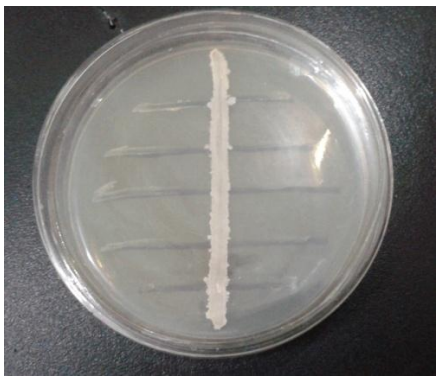

Fig. 1. A cross Streak plate of *Bacillus* sp. shown to have antimicrobial activity against *Escherichia coli* (clinical isolate).

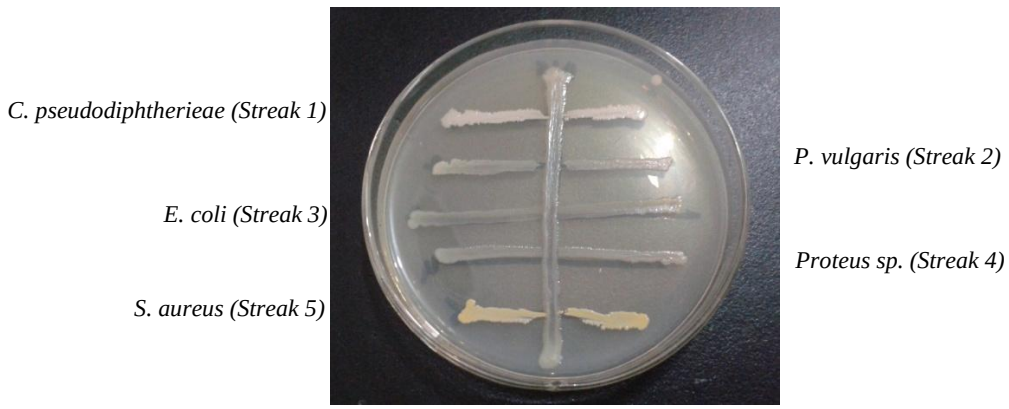

Fig. 2. A cross streak plate of *E. coli* shown antimicrobial activity against *C. pseudodiphtherieae* (streak 1), *Pseudomonas vulgaris* (streak 2) *and Staphylococcus aureus* (streak 5).

It was reported that bacterial strains isolated from soil are the potential producers of antibacterial agents (Singh *et al*, 2012, Zia-ul-Haq *et al*, 2011). A number of organisms have been isolated but *Bacillus* species are of considerable importance because they produce a number of antibiotics like chloramphenicol, erythromycin, neomycin, nystatin (Waites et al, 2008). *Bacillus* species produces many kinds of antibiotics which share full range antimicrobial activity such as bacitracin which is produce by *Bacillus licheniformis* is a mixture of at least

5 polypeptides. These antibiotics are reported to be active against many of the Gram positive organisms such as *Stapylococci, Streptococci*, etc. but they are not as active against most of the Gram negative bacteria (Mc Evoy, 1993). One of the isolated *Bacillus* sp. is active against Gram negative organism. The soil microbial isolates were showing antimicrobial activity under normal growth conditions and was found inhibiting both the gram positive and gram negative organisms. The present study is a preliminary work with potential findings which could be elaborated in future to produce novel isolates and antibiotics.

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