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Screening of Antimicrobial Activity of Bacillus Species Isolated From Soil

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ABSTRACT

Most antibiotics used today for the treatments of infectious diseases caused by pathogenic bacteria are mostly isolated from microbes and the increase in emergence of resistance need broad spectrum antibiotics are required nowadays. Aim of this study is the extraction of new antibacterial compounds from soil samples and determination of their efficacy against human pathogenic bacteria. The present study was designed to screened antibiotic production by *Bacillus species*, supernatant of isolates was taken and antibacterial activity was tested against gram positive bacteria including *S. aureus*, *Micrococcus* and gram negative bacteria including *Pseudomonas, E.coli* and *Proteus* by using agar well diffusion method. Isolated strain shows zone of inhibition against *S. aureus*, *Micrococcus*, *E.coli*, and *Proteus*. No zone of inhibition has been shown against Pseudomonas. Biochemical analysis was performed for the identification of *Bacillus species*. For further investigation, identification of strains may be helpful in improving antibiotic production.

Keywords: *Antimicrobial activity, Bacillus, screening, soil*

INTRODUCTION

Human pathogenic bacteria are one of the most serious threats to man's health (Ali and Rahman, 2014). Treatment of these dangerous, infectious diseases which are caused by pathogenic bacterial strains is one of the most critical problems in the clinical field. This necessity persuaded the investigators to synthesize new, novel and more potent inhibitory compounds (like azoles and quinolones derivatives) to fight these diseases. However, the increase in the rate of resistance of bacteria in recent few years, is due to increased use of commercially available antibiotics. Because of this reason investigators move toward the study on natural products from microorganisms to discover new and safe lead compounds.To reach this approach, it is must to screening of bacterial strains which were able to produce inhibitory compounds (Moshafi, 2011). Another one is this antagonistic microorganism's produce a wide variety of antimicrobial metabolites (Han and Shim, 2015) and product of these metabolites were also used in antibiotic production (Ali and Rahman, 2014).

Phylogenetically, bacteria belonging to genus of *Bacillus* are one of the most abundant bacterial strains which were able to produce scores of antibiotic compounds with several of different chemical properties, among these peptide derivatives are the most studies (Moshafi, 2011). Ability of *Bacillus* species to survive and grow in different ecosystems is based on their potential to produce endospores. They exhibit diverse physiological properties that enable and are capable to degrade many substrates such as cellulose, starch, pectin, proteins and hydrocarbons and so on (Nicholson, 2002). The genus *Bacillus* and Clostridium can be found in two distinct states. In vegetative state, they are metabolically active and use all available nutrients that promote their growth. In contrast, when nutrients are depleted, a developmental program of endospores formation (sporulation) is initiated, resulting in the production of highly resistant spores. They undergo dormancy (Piggot and Coote, 1976). In liquid medium, sporulation is

usually triggered by starvation of a carbon or nitrogen source, and sometimes by phosphate starvation. Two methods are generally used. First, is an exhaustion procedure, whereby bacteria grow in the medium, use up some essential nutrient and then sporulate. Secondly, by a replacement technique where bacteria are growing exponentially in a nutrient rich medium are transferred in to a nutrient poor medium. Many enzymatic activities increase during sporulation like exoprotease activity produced by *B. subtilis* (Errington, 2003). After the initiation of sporulation, antibiotic production is also among one of the earliest events that have been developed (Al-Saraireh *et al.*, 2015.)

With the progression of molecular techniques development, especially by cloning, molecular screening and protein engineering, the greater speed and accuracy in cloning enzyme genes from microorganisms and way to generating versions with improved properties (Good fellow and O'Donnell, 1993). Secondary metabolites also called as idiolites, are special compound also possessing chemical structure but are different from primary metabolites. The production of secondary metabolites during their growth is not essential for growth but have other diverse functions in nature. These metabolites produce by some genera and some species of that genus, played a critical role in the discovery and advancement of many antibiotics. Soil corresponds to a potential habitat for discovering and isolating new natural products (Sansinenea and Ortiz, 2011). *Bacillus* species produce secondary metabolites during their stationary growth phase. Secondary metabolites have wide structural diversity by which microbial extract were made in industries and screening procedures of these microbial extracts disclose vast structural variations in natural compounds. By producing these metabolites the bacteria are able to compete with them in a natural environment. These findings increase the potential industrial importance of *Bacillus* spp., particularly of B. thuringiensis, for insecticidal usage (Drobniewski, 1993).

Bacteriocins are ribosomally synthesized bacterial peptides and protenacious toxins which inhibits or kill the other microbes. Bacteriocins produced by Gram positives organism have developed some great interest because of their potential use in food preservation, they also act like a therapeutic agents against Gram positive and negative bacteria. More importantly in modifying the role of gut microflora. *Bacillus* species includes B. thuringiensis, *B. subtilis*, *B. stearothermophilus*, *B. licheniformis*, *B. megaterium*, and *B. cereus* have been reported to produce bacteriocins and also the closely related substances. Among these antibiotics, Nisin and Lanthibiotic are widely used as a food preservative (Abriouel *et al,* 2011). Antibiotics are called as the wonder drugs for their success against pathogenic organisms. Remarkable groups of these compounds form a heterogeneous assembly of biologically active molecules with different structure and modes of action. They have attacking power virtually against every type of microbial activity such as DNA, RNA and protein synthesis, electron transport chain, endospores formation and many others. Antibiotics produce by *bacillus* species are polypeptides in nature and are low molecular weight compound that are synthesized by ribosomal and non ribosomal mechanism. (Mannanov and Sattarova, 2001) Antibiotics such as gramicidin, tyrocidine, and bacitracin are synthesized non ribosomally by the multi enzymes thio template mechanism. Surfactin and mycobacillin like substances are also synthesized non ribosomally but by a mechanism distinct from that of the multi enzyme thiotemplate mechanism. Other antibiotics such as subtilin are gene encoded and are synthesized ribosomally (Nakano and Zuber, 1990). The names "gramicidin" and "gramicidin D" are often used for a linear

polypeptide antibiotic complex isolated from *Bacillus brevis* by Dubos. In contrast Gramicidin is called a cyclic peptide. The commercially produced gramicidin actually has several forms; the major component is gramicidin A. Gramicidin is capable of transporting ions through biological membranes. It has been notify that gramicidin involved in the regulation of bacterial sporulation and a specific inhibitor of enzyme RNA polymerase that affects the binding of RNA polymerase to DNA. In vitro gramicidin may also affect DNA supercoiling (Marahier *et al.*, 1993). Gramicidin S, produce by *Bacillus brevis*, a cyclic deca peptides formed during the stationary phase cultures of its growth. It is polypeptides with greater content of reactive group and complex amino acid. The Tyrocidine itself is a mixture of four cyclic deca peptides, such as tyrocidines A, B, C, and D, in which Phenylalanine and Tyrosine residues are gradually replaced by Tripsin, this replacement of residue is dependent on the relative concentrations of these amino acids in the growth medium (Henning *et al.*, 1997). It is a mixture of cyclic peptides produce by *Bacillus* species *B. licheniformi*, and first discovered in

1945 and mostly use for topical application, it is another non ribosomal synthesizes antibiotic (NRPSs), which mean that ribosome are not involve in its synthesis (Schmidt, 2004). Bacitracin divided into 3 classes as bactericin or bac A,B,C (Stone and Strominger 1971).

An increased abundance of β-Lactamase is a frequent source of antibiotic resistance in bacteria. Many of *Bacillus* species also induce B-Lactamase genes that are found in their wild-type genomes (Fenselau *et al.*, 2008). It has been reported that *Bacillus anthracis* is susceptible to a limited number of antimicrobial agent; it has also been noted that they are resistance to penicillin, erythromycin and quinolones (Luna. *et al.*, 2007).

This recent study was designed to investigate and identifY *Bacillus* species from soil and evaluates the production of antibiotics and antimicrobial testing against some pathogenic bacteria. Now, thousand of antibiotics have been discovered but only few of them are useful to human and animal the major reason behind this is the toxicity of antibiotic. In order to get over these problem search of new more effective antibiotic such as from natural resources or from bacteria, is in progress which also does not have any toxic effect or side effect.

MATERIALS AND METHODS

Sample Collection: The different garden soil sample were collected from Karachi in a sterile polythene bags and brought to the laboratory of microbiology in JUW Karachi.

Sample Preparation: 1g of soil weighted and transfers to tube containing 5ml nutrient broth and placed in an incubator at 37°C for 24 hours.

Isolation and Identification of *Bacillus* ***Species:*** After overnight incubation, tubes were removed from incubator, transfer 0.1 ml supernatant form the tube, then inoculated in nutrient agar plates by four way streaking and again incubated at 30°C for 24 hours. After the subsequent incubation, plates were examined for determination of colonial morphology and the suspected colonies of *Bacillus* were identified by staining techniques. Gram-positive spore forming rods were selected for further biochemical characterization as described by the Bergey's Manual.

Antimicrobial Crude Preparation from *Bacillus* ***Species:*** Each isolate was grow in Brain Heart Infusion (BHI) broth and incubated the broth overnight at 37°C with shaking water bath.

The cells were collected by centrifugation at 10000xg for 20 minutes. The supernatants were obtained after centrifugation is filtered through filter paper. Crude obtained was preserve in sterile flask at 4°C till further use

for antimicrobial susceptibility testing.

Antimicrobial Activity Testing: Antimicrobial testing of crude obtain from *Bacillus* species was tested against different pathogenic bacteria including *S. aureus*, *Pseudomonas*, E.col, *Micrococcus*, *Proteus* and it was tested by performing agar well diffusion method. Total of five organisms were spread on Mueller Hinton Agar after standardization the culture with 0.5 McFarland turbidity tubes. Wells were made for diffusion by using sterile 2cm metal borer in all plates then transfer 100µl crude in all wells by justers. Keep the plates for 1 hour at room temperature. Plates were incubated for overnight at 37°. Next day, results were observed and was determined the antimicrobial activity of *Bacillus* species.

RESULTS

Isolation of *Bacillus* species was done by collecting 10 different garden soil samples of Karachi and colonies of *Bacillus* was observed on nutrient agar plate. The colonies of *bacillus* shows whitish creamy, dry, flat and irregular margin with sticky consistency. When observed under the microscope they appear as gram positive rods in the arrangement of chains and endospores were also observed (as shown in fig: 6). the identification tests for *bacillus* species are discussed in table.1. *Bacillus* species isolated from different garden soil samples were *B. subtilis* and *B. cereus*. From these strains the obtained crude shows antibacterial activity against pathogenic organism, that are *S. aureus*, E. coli, *Micrococcus*, *Proteus* which seen by zone of inhibition around colonies. Both *Bacillus*

Table I. Biochemical characteristics of Bacillus species

Test	S#1	S#2	S#3	S#4	S#5	S#6	S#7	S#8	S#9	S#10
Nitrate	+	+	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+	+	+
Iodole	–	–	–	–	–	–	–	–	–	–
MR	–	–	–	–	–	–	–	–	–	–
VP	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
Coagulase	+	+	+	–	–	+	–	–	+	–
Mannitol without gas	+	+	+	–	–	+	–	–	+	–
Bacillus species	*B. subtilis*	*B. subtilis*	*B. subtilis*	*B. cereus*	*B. cereus*	*B. subtilis*	*B. cereus*	*B. cereus*	*B. subtilis*	*B. cereus*

Table II. Antibacterial Activity against test organisms:

Test Organisms	Antibiotic crude obtain from Bacillus species from different samples									
	1	2	3	4	5	6	7	8	9	10
S. aureus	+	+	–	+	–	–	++	–	–	+
Pseudomonas	–	–	–	–	–	–	–	–	–	–
Micrococcus	–	+	+	++	+	+	++	+	–	+
Proteus	++	++	+	+	++	+	+	–	++	+
E. coli	+	++	+	+	++	+	++	++	+	++

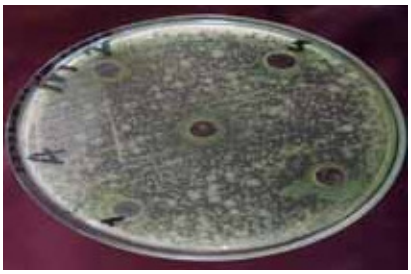

Figure 1. Activity of Bacillus species against *Micrococcus sp.*

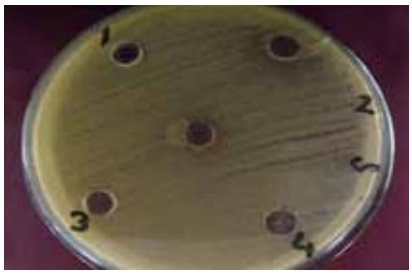

Figure 2. Activity of Bacillus species against *S.aureus*

Figure 3. Activity of Bacillus species against *Pseudomonas sp.*

Figure 4. Activity of Bacillus species against *E. coli*

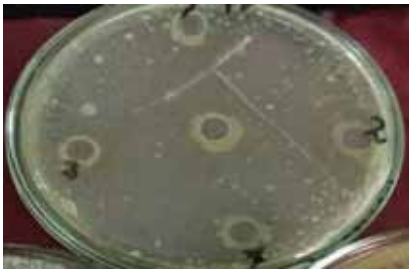

Figure 5. Activity of Bacillus species against *Proteus*

species shows no activity or zone of inhibition against *Pseudomonas*. These results are shown in table 2 and in fig: 1, 2, 3, 4, and 5.

DISCUSSION

Many species of *Bacillus* are of great importance because of their ability to produce antibiotics. Screening of these new antibiotics from natural resources and from microorganism has becoming greatly important for pharmaceutical industry. Pathogenic microbes showed increase resistance against used therapeutic agents.

The current study was carried out to check production of antibiotic by *Bacillus* species isolated from soil.

Antimicrobial activity was tested by performing agar well diffusion method. Observed results revealed that isolated strains of *Bacillus* (*B. subtilis* and *B. cereus*) have potential of producing antibiotic substances. It was determined that crude obtain from *B. subtilis* and *B. cereus* has an inhibitory effect against gram positive organism including *Micrococcus* and *S. aureus* but the effect was more pronounced against *Micrococcus* as compare with *S. aureus*. Among gram negative bacteria including E. coli, *Pseudomonas* and *Proteus*, the crude that were obtained prove to be more effective on E.coli in contrast to *Proteus*. *Pseudomonas* were shown as resistant to all crude obtain from both *Bacillus* species because of highly resistance gene presences or by presence of efflux pumping mechanism and due to *Pseudomonas* also produce bacteriocin which known as pyocine.

Aslim *et al* in year 2002 determined that strains of *Bacillus* had a tremandous activity against Gram-positive bacteria as compare to Gram-negative bacteria (Aslim *et al*., 2002). Furthermore Nakano and Zuber in 1992 reported that *B. cereus* has inhibitory effects against Gram-positive and Gram-negative bacteria (Nakano and Zuber, 1990). All these studies and regarding to the recent study, results were confirmed and it has been concluded that *Bacillus* species which were isolated from soil samples possessed antibacterial activity against Gram-positive and Gram-negative pathogenic bacteria.

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