A peer-reviewed journal
PSM Microbiology
2016 | Volume 1 | Issue 1 | Pages 01-04
ISSN: 2518-3834 (Online)

www.psmpublishers.orgs

Research Article **Open access**

Isolation and Screening of Antibiotic producing Bacteria from Soil in Lahore City

Fakhar-un-Nisa Yunus[1*], Zam Zam Khalid[1], Farzana Rashid[1], Asfa Ashraf[1,3], Muhammad Naeem Iqbal[2,3], Firasat Hussain[4]

[1]Department of Zoology, Lahore College for Women University, Jail Road Lahore 54000, Pakistan.
[2]The School of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China.
[3]Pakistan Science Mission (PSM), Noor Kot 51770, Pakistan.
[4]Yunnan Institute of Microbiology, Yunnan University, Kunming, 650091, PR China.

Received: 10.Jan.2016; Accepted: 17.May.2016; Published Online: 18.Jul.2016
***Corresponding author: Muhammad Naeem Iqbal; Email: driqbalnaeem@hotmail.com**

Abstract
The need for new antibiotics is increasing with the increase in antibiotic resistance. The present research work has been focused on the isolation of antibiotic producing bacteria from the soil samples collected from Jinnah garden, Lahore. The isolates were identified based on their morphology and further confirmed through biochemical tests. Antibiotic producing ability was confirmed by inhibition zones around bacterial colonies. A total of 56 isolates belonging to four different strains of antibiotic producing bacteria were found in different soil samples. Out of these, 30 were *Bacillus subtilis*, 9 were *Bacillus licheniformis*, 12 were *Streptomyces* and 5 were *Actinomycetes*. A significant number of antibiotic producing bacteria were found in this study. These results suggest that soil isolates, having antibiotic producing capability can be used commercially after proper standardization.
Keywords: Screening, Isolation, antibiotics production, bacteria.

To cite this article: Yunus, F.N., Khalid, Z.Z., Rashid, F., Ashraf, A., Iqbal, M.N., Hussain, F., 2016. Isolation and Screening of Antibiotic producing Bacteria from Soil in Lahore City. PSM Microbiol., 01(1): 01-04.

INTRODUCTION

Antibiotics can be defined as any chemical substance of small organic molecules which at low amounts are harmful to the metabolic activities and growth of other microbes (Brun and Skimkets, 2000). The natural products obtained from microorganisms are still appearing as the most auspicious source of the future antibiotics (Pelaez, 2006). Antibiotics which are mostly in use today are the natural derivatives of fungi and actinomycetes (Tawiah *et al.,* 2012). In nature, there is universal dissemination of antibiotics among the microorganisms responsible for their antagonism (Euanorasetr *et al.,* 2010).

There are numbers of bacteria which are capable of producing antibiotics which include *Bacillus* (Waites *et al*., 2008), *Actinomycetes* (Abdulkadir and Waliyu, 2012; Tiwari and Gupta, 2013) *Pseudomonas* (Cartwright *et al*., 1995), and *Streptomyces* (Willey *et al*., 2008) and most of these isolates are soil bacteria.

Extensive use of antibiotics both for clinical and veterinary purposes leads to the development of resistance in many infectious strains (Kurtboke *et al*., 1992). With the continuation of this process of resistance, the search for new anti-infective drugs should be carried out (Thakur *et al.,* 2007; Brown and Wright, 2005). In addition to the resistance problem, a number of new infectious diseases have been discovered over the past 30 years (Zimmerman and Zimmerman, 2003; Bryskier, 2005).

Soil is rich in microorganisms which are capable of producing antibiotics (Brun and Skimkets, 2000). The traditional approach is 'random screening' in which bacteria are isolated, grown and their activity spectrum was assessed. Even this has been done for more than 50 years still we are getting results in favor to us and thus we are sticking with this approach (Wawrik *et al.,* 2007).

The aim of this study was isolation and screening of antibiotic producer bacteria from soil samples from the Jinnah garden, Lahore.

MATERIALS AND METHODS

Collection of samples

A total number of 25 soil samples weighing approximately 10 gram each were collected from different areas of Jinnah garden, Lahore. All the samples were collected 4 to 5 cm deep from the surface using spatula

and placed in sterilized zip lock polythene bags. The samples were transferred to Zoology Research Laboratory, Lahore College for Women University and kept at 4°C for further processing.

Processing of samples

Samples were processed by dissolving 1 g of soil in 10 ml of sterile distilled water to make soil suspension in test tube. The test tube was vortexed for 2 to 3 minutes to remove soil, stones and debris. Supernatant was transferred to another test tube and ten-fold serial dilutions were prepared. Then spread 100µl of supernatant from each dilution on nutrient agar plates and kept at 37°C overnight. After 24 to 48 hrs, zone of inhibition in each plate was observed and colonies were selected for further confirmation of isolates.

Isolation and Screening of bacteria

Selected colonies based on morphology and zone of inhibition were plated directly onto nutrient agar for isolation of bacteria. After an overnight incubation, discrete colonies of bacteria were selected based on colony characters and streaked on new agar plates according to previous study (Iqbal *et al.,* 2015). The plates were then kept at 37°C for 24 hrs.

Multiple streak plate method as used previously (Iqbal *et al.,* 2015) was applied to obtain purified cultures of bacteria on nutrient agar plates. Pure single colonies were noticed after 24 hrs incubation at 37°C.

Purified cultures of bacteria were identified based on colony morphology, microscopic characters and biochemical characters (Iqbal *et al.,* 2015; Yunus *et al.,* 2016; Yunus *et al.,* 2016) following Bergey's Manual of Determinative Bacteriology (Bergey, 1984).

Statistical Analysis:

Data obtained was arranged using Microsoft Excel (Microsoft Corporation). Statistical difference between means was determined by ANOVA using SPSS version 16.0 at $p<0.05$ significance level.

RESULTS

A total of 56 bacterial fifty six isolates having zones of inhibition on crowded plate have been isolated and identified by morphological characteristics and biochemical tests from 25 soil samples (Table 1). Four different bacterial strains were present, which were *Bacillus subtilis*, *Bacillus licheniformis*, *Streptomyces* and *Actinomycetes* species. Out of these 56 isolates 30 were *Bacillus subtilis*, 9 were *Bacillus licheniformis*, 12 were *Streptomyces* and 5 were Actinomycetes (Table 1). Production of antimicrobial compounds was confirmed by inhibition of other bacterial growth around the colonies (Figure 1).

One way ANOVA was applied to compare means of various isolates of antibiotic producing bacteria. The bacterial isolates showed significant values ($p<0.05$) in soil samples (Table 2).

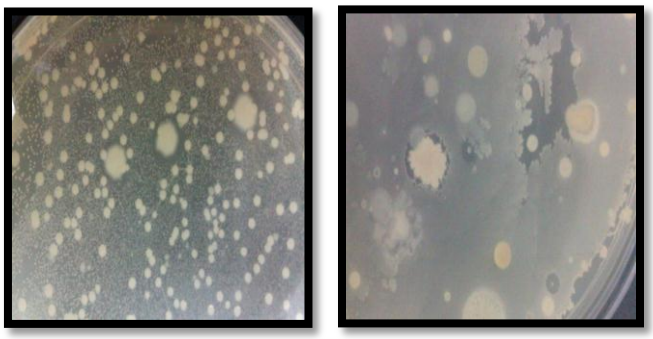

Fig. 1. Microbial colonies with zones of inhibition on crowded plate

Table 1. Morphological and biochemical identification of antibiotic producing bacterial isolates

Bacterial Morphology	Colony Morphology	Gram Staining	Spore Staining	Casein Decomposition	Catalase Test	Gelatin test	Acid Production Test	Indole test	Oxidase Test	Starch Hydrolysis	Identified strains
Rods	Dry, flat, irregular, lobate margins, creamy white colonies spread from the center	+ive	+ive	+ive	+ive	+ive	-ive	-ive	+ive	+ive	*Bacillus subtilis*
Rods	Rounded to irregular colonies, undulate to fimbricate margins, white, attach strongly to the agar	+ive	+ive	+ive	+ive	+ive	-ive	-ive	+ive	+ive	*Bacillus licheniformis*
Filamentous	Chalky, white colonies, hard, dusty, attached firmly to the agar	+ive	-ive	+ive	+ive	+ive	-ive	-ive	-ive	+ive	*Streptomyces* spp.
Filamentous rods	Branching filaments, pinpoint colonies, convex	+ive	-ive	+ive	+ive	+ive	+ive	-ive	-ive	+ive	*Actinomycetes* spp.

Table 2. One way Analysis of Variance showing significant values (p<0.05) of bacterial isolates

Type of bacteria	No. of Samples	No. of Isolates	Mean X	Standard Deviation STD	Standard Error SE	Remarks
Bacillus subtilis	25	30	1.2	1.224	0.244	Significant
Bacillus licheniformis	25	9	0.36	0.637	0.127	Significant
Streptomyces	25	12	0.48	0.822	0.164	Significant
Actinomyces	25	5	0.20	0.408	0.081	Significant

DISCUSSION

Soil samples are commonly evaluated for isolation of the antibiotic producing organisms, because soil microorganisms produce lots of antibiotics in order to survive in such a competitive environment. Bacteria producing high number of medically and agriculturally important antibiotics belong to genera *Bacillus*, *Streptomyces* and *Pseudomonas* (Yoshiko *et al*., 1998; Sharga *et al*., 2004).

As the 39 isolates from 56 were *Bacillus* species and 9 isolates were *Bacillus licheniformis,* these results correlates with many previous studies that *Bacillus* spp are notorious producing antibiotics. It has been documented that *Bacillus* genus and other spore forming bacteria carry genes for the breakdown of diverse carbon source and production of antibiotics (Prescott *et al.,* 2008). *Bacillus* species are ubiquitous in nature. Many stains of the genus *Bacillus* have capability to synthesize a wide range of antibiotics. Hassan et al. (2014) reported 14 isolates of antibiotic producing Bacillus species from soil samples.

Several hundred *Bacillus subtilis* strains have been described with the ability to synthesize more than two dozen antibiotics with diverse structures. The potential of *Bacillus subtilis* to synthesize antibiotics has been perceived for 50 years. The large number of antibiotics produced from *Bacillus subtilis* might reflect the diversity of natural isolates. Other *Bacillus* species like *Bacillus amyloliquefaciens* (Koumoutsi *et al.,* 2004)*, Bacillus brevis* produce few antibiotics compared to *Bacillus subtilis*.

Few antibiotics producing Actinomycetes have been isolated in the present work. The reason why only small number of Actinomycetes has been isolated may be the textures of soil and other predominant environmental conditions at the study location. Actinomycetes require a longer time to grow compared to other bacteria as it has been documented by previous studies (Ahmed *et al*., 2013). Actinomycetes can use a variety of organic nutrients but special media are often preferable (Rahman *et al*., 2000; Sultan *et al*., 2002). Probably this could be the logic why Actinomycetes grow in very small number on the media (Nutrient agar) used. Tryptic soy agar or Starch-Casein agar medium are mostly used for the isolation of Actinomycetes in many literatures.

Streptomyces are the most studied and well known group of Actinomycetes. These are gram positive, filamentous bacteria which have a great capability to synthesize most important secondary metabolites such as antibiotics, antitumor, antivirals and antifungals (Dehnad *et al.,* 2010). Out of 56 isolates, 12 were *Streptomyces* having white, wrinkled colonies. Although, various biochemical tests were performed but the strains were not identified to the species level. For proper identification of genera and species of Actinomycetes as well as morphophological and physiological characteristics, different other biochemical parameters such as cell wall chemo type, peptidoglycan type, whole cell sugar pattern, phospholipid type and G+C% of DNA should be considered (Dehnad *et al.,* 2010).

CONCLUSION

It is concluded that *Bacillus subtilis*, *Bacillus licheniformis*, *Streptomyces* and *Actinomycetes* isolated from Jinnah garden, Lahore have potential to produce antibiotics.

ACKNOWLEDGEMENT

The authors are highly thankful to Dr. Fakhar-un-Nisa Yunus, Department of Zoology, Lahore College for Women University, Lahore, for technical support during this research work.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

REFERENCES

Abdulkadir, M., Waliyu, S., 2012. Screening and Isolation of the Soil Bacteria for Ability to Produce Antibiotics. Eur. J. Appl. Sci., 4(5): 211-215.

Ahmed, R.N., Sani, A.H., Ajijolakewu., Alamu, F.B., 2013. Soil Screening for Antibiotic - Producing Microorganisms. Adv. Environ. Biol., 7(1): 7-11.

Bergey, S.A., 1984. Bergey, Manual of Determinative Bacteriology, 9th edition, Williams & Wilkins., Philadelphia.

Brown, E.D., Wright, G.D., 2005. New Targets and Screening Approaches in Antimicrobial Drug Discovery. Chem. Rev., 105: 759-74.

Brun, Y.V., Skimkets, L.J., 2000. Prokaryotic development. ASM Press, Pp. 11-31.

Bryskier, A., 2005. In Pursuit of New Antibiotics. In: Antimicrobial Agents. ASM Press, Washington, D.C. Pp. 1242-59.

Cartwright, D.K., Chilton, W.S. Benson, D.M., 1995. Pyrrolnitrin and phenazine produce by *Pseudomonas cepacia*, strain 5.5 B, a biological agent of Rhizoctonia solani. Appl. Microbiol. Biotechnol., 43: 211-121.

Dehnad, A.R., Yeganeh, L.P., Bakhshi, R., Mokhtarzadeh, A., Soofiani, S.A., Monadi, A.R., Gasanova, S., Abusov, R., 2010. Investigation antibacterial activity of Streptomycetes isolates from soil samples, West of Iran. Afr. J. Microbiol. Res., 4(16): 1685-1693.

Hassan, S.A., Hanif, E., Zohra, R.R., 2014. Isolation and screening of soil bacteria for potential antimicrobial activity. FUUAST J. BIOL., 4(2): 217-219.

Iqbal, M.N., Anjum, A.A., Ali, M.A., Hussain, F., ALI, S., Muhammad, A., Irfan, M., Ahmad, A., Irfan, M. and Shabbir, A., 2015. Assessment of microbial load of un-pasteurized fruit juices and in vitro antibacterial potential of honey against bacterial isolates. Open Microbiol. J., 9: 26-32. DOI: 10.2174/1874285820150601E001.

Koumoutsi, A., Chen, X.H., Henne, A., Liesegang, H., Hitzeroth, G., Franke, P., Vater, J., Borriss, R., 2004. Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive cyclic lipopeptides in Bacillus amyloliquefaciens strain FZB42. J. Bacteriol., 186(4):1084-96.

Kurtboke, D.T., Chen, C.F., William, S.T., 1992. Use of polyvalent phage for reduction of *Streptomyces* on soil dilution plates. J. Appl. Bacteriol., 72: 103.

Pelaez, F., 2006. The historical delivery of antibiotics from microbial natural products_Can history repeat? Biochem Pharmacol*.,* 71(7): 981-990.

Prescott, M.L., Harley, P.J., Klein, A.D., 2008. Microbiology. 7th Ed. Publishing Group, Pp. 42-51, 232-233, 762-764.

Rahman, M.Z.A., Azam, A.T.M.Z., Gafur, M.A., 2000. *In vitro* antibacterial principles of two flavonoids and extracts from *Clerodendrum indicum* linn. Pak. J. Biol. Sci., 3(10): 1769-1771.

Sharga, B.M., Nikolaychuk, V.I., Maga, M.I., 2004. Comparative IR Spectrometry of antimicrobial substances derived antibiotic from *Bacillus*. Mikpobiojiotir., 15: 75-77.

Sultan, M.Z., Khatune, N.A., Sathi, Z.S., Bhuiyan, M.S.A., Sadik, M.G., Choudury, M.A., Gafur, M.A., Rahman, M.A.A., 2002. *In vitro* antibacterial activity of an active metabolite isolated from *Streptomyces* species. Biotechnol., 1(2-4): 100-106.

Tawiah, A.A., Gbedema, S.Y., Adu, F., Boamah, V.E. and Annan, K. (2012). Antibiotic producing microorganisms from River Wiwi, Lake Bosomtwe and the Gulf of Guinea at Doakor Sea Beach, Ghana. BMC Microbiology, 12: 234-241.

Thakur, D., Yadav, A., Gogoi, B.K., Bora, T.C., 2007. Isolation and screening of *Streptomces* in soil of protected forest areas from the state of assam and tripura India for antimicrobial metabolites. J. Mycol Med., 17: 242-49.

Tiwari, K., Gupta, R.K., 2013. Diversity and isolation of rare actinomycetes: an overview. Crit. Rev. Microbiol., 39(3): 256-294.

Waites, M.J., Morgan, N.L., Rockey, J.S., Higton, M., 2008. *Industrial Microbiology-an Introduction*. Blackwell Publisher, London.

Wawrik, B., Kutliev, D., Abdivasievna, U.A., Kukor, J.J., Zylstra, G.J. Kerkhof, L., 2007. Biogeography of Actinomycetes communites and type II polyketide synthase genes in soils collected in New Jersey and Central Asia. Appl. Environ. Microbiol., 73: 2982-9.

Willey, H.J., Linda, P.S., Shewood, M., 2008. Microbiology. 5th Ed. McGraw Hill Publisher, London. P. 1035.

Yoshiko, H., Okamoto, S., Muramatsu, H.. Ochi, K., 1998. Acquisition of certain Streptomycin resistant Mutations Enhances antibiotic production in bacteria. Antimicrob Agents Chemother., 42(8): 2041-2047.

Yunus, F.N., Kanwal, F., Rashid, F., Ashraf, A., Iqbal, M.N., Xiao, S., 2016. A Comparative Study on Isolation and Identification of *Bacillus thuringiensis* from Different Localities of Gujranwala City. PSM Biol. Res., 01(1): 34-38.

Yunus, F.N., Iqbal, M., Jabeen, K., Kanwal, Z., Rashid, F., 2016. Antagonistic activity of *Pseudomonas fluorescens* against fungal plant pathogen *Aspergillus niger*. Sci. Lett., 4(1): 66-70.

Zimmerman, B.E., Zimmerman, D.J., 2003. Microbes and Diseases That Threaten Humanity. Contemporary Books. Chicago: New York, San Francisco.

