International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

IJAMSCR | Volume 2 | Issue 2 | April-June-2014
www.ijamscr.com

Research article

Bioactivity Screening of Soil Bacteria against Human Pathogens

Loganathan Veerappan[*1], Vinoth S[2], Thirunalasundari T[2]

*****School of Pharmacy, Taylor's University, 47500 Subang Jaya, Selangor, Malaysia.

[2]Department of Biotechnology, Bharathidasan University, Tiruchirapalli, India.

ABSTRACT

Microorganisms have a profound effect on medical science as they not only infect & cause disease but also produce metabolic products that can cure infections. Soil happens to be a source for a variety of microorganisms. Most of the bacteria, particularly actinomycetes produce biologically active secondary metabolites. Though there are a number of antibiotics available, there is a pressing need for the discovery of new source for antimicrobials against the pathogens due to the development of drug resistance of the pathogenic microorganisms. In addition to, new pathogenic strains are also developing and causing infection to human beings. Bioactive compounds are compounds that are produced by any living organism and are known to exhibit various biological activities both *in-vitro* & *in-vivo*. Bioactivity may be antimicrobial, antineoplastic, anticancerous, immunomodulation, antifertility & others. Soil bacteria were isolated by standard technique and by making use of selective media. The isolates were identified and subjected for preliminary screening to look for their ability to produce bioactive materials. A total of 36 strains were isolated from three different soil samples. 14 of them were found to have antibacterial activity against the human pathogens like *Staphylococcus aureus, Streptococcus faecalis, E.coli, Klebsiella aerogenes, Proteus vulgaris, Pseudomonas aureginosa* and *Salmonella typhi* by preliminary screening. Further the selected (3) bacteria were grown in the suitable culture media for the production of bioactive metabolites by using rotary shake flask. The active metabolites was isolated by solvent extraction and concentrated by evaporation under reduced pressure. The antimicrobial screening of the active metabolites showed prominent effect against the clinical pathogens under the study.

Key Words: Soil bacteria, Antibacterial, Human pathogens.

INTRODUCTION

The biological diversity of microorganisms is mainly due to the diversity not only in their morphology but also in their role. There exists an antagonistic effect between each and every organism, because of their tendency to produce biologically active compounds. Bioactive compounds are compounds that are produced by any living organism and are known to exhibit various biological activities both *in-vitro & in-vivo*. Bioactivity may be antimicrobial, antineoplastic, anticancerous, immunomodulation, antifertility & others. Soil and sewage are considered to contain most of the nutrients required for the survival of almost all microorganisms, which are potential enough to produce antibiotic compounds. Literature reveals the production of a new peptide antibiotic named thioxamycin, isolated from the culture broth of *Sterptomyces sp*.[1] It is active against anaerobic gram positive and gram negative bacteria and also aerobic gram positive bacteria by *in-vitro* analysis. Cystargin is a new antifungal agent produced by *Kitosatosporia cystarginea*.[2] A novel antitumor compound chrolactomycin, isolated from *Sterptomyces sp.* 569N-3, which exhibited antimicrobial activity and anti proliferative activity against human tumor cell lines.[3] Having known these facts an attempt was made in this study to look for new microbe with a potential to produce novel antimicrobial.

MATERIALS AND METHOD

Isolation of soil microbes

A total of three different soil samples were collected from three different locations of Tiruchirapalli city where garbage & other waste being dumped. The samples were shade dried to remove the moisture. All the three soil samples were subjected for physicochemical analysis like colour, texture, pH and moisture content and biological analysis by standard methods. Known quantity of the sample was weighed and used for isolation of active bacteria by making use of plate culture technique.[4] A few selected individual colonies differing in their size, morphology and color were isolated and subcultured on separate slants. The bacterial isolates were characterized in terms of their morphology and staining characteristics using grams stain technique.

Screening of soil isolates for their anti bacterial activity

The soil isolates were subjected for preliminary screening for the production of antibacterial compounds by making use of cross streak technique against standard and clinical strains of bacteria.[5] The standard strains like *Staphylococcus aureus* NCIM 2079 , Streptococcus faecalis NCIM 2080 and E.coil NCIM 2065, *Klebsiella aerogenes* NCIM 2098, *Proteus vulgaris* NCIM 2027, *Pseudomonas aureginosa* NCIM 2036 were obtained from National Chemical Laboratory, Pune and the clinical strains of gram positive bacteria like *Staphylococcus aureus, Streptococcus faecalis* and gram negative organisms like *E.coli, Klebsiella aerogenes, Proteus vulgaris, Pseudomonas aureginosa, Salmonella typhi* were obtained from Kings Institute of Preventive Medicine, Guindy, Chennai -32 and were used for initial screening.

RESULTS

A total of three different sites were selected for collection of soil samples where the soil microorganisms get exposed to different environmental stress. The soil samples were coded as S1, S2 and S3. Table-1 reveals the physicochemical nature of the soil samples. The physical nature of the soil samples S1 & S2 were found to be fine powder and S3 was granular in nature. S1 and S3 were being slightly alkaline to neutral and the sample S2 was slightly acidic to neutral. Table-2 reveals the biological nature of the soil samples. The soil samples were found to have microbes like bacteria, fungi, algae, nematodes and worms.

A total of thirty six different bacterial strains were isolated from the soil samples and were used to look for their antibiotic producing capability by preliminary screening against different standard strains and clinical pathogens. Fourteen were found to be highly active against more than two pathogens used in this study and were noted as bioactive isolates.

A total of seven isolates showed activity against *Staphylococcus aureus* and the other 29 remained inactive (Table -3). Six isolates showed activity against *Streptococcus feacalis* and 30 did not. Seven isolates were found to have activity against the clinical strain *E.coli*. *Klebsiella aerogenes* was restricted by six isolates. *Pseudomonas aureginosa* was dominant against as many as 32 isolates and only four of the isolates showed activity against it. *Protease vulgaris* was restricted by six isolates whereas *Salmonella typhi* showed activity against nine isolates.

DISCUSSION

Though there are a number of antibiotics available, there is a need for discovery of new source for antimicrobials. The reason is the development of multidrug resistance of the existing pathogens and also development of newer strains to cause infections. Here the soil is selected as a source of microorganism which could produce biologically active compounds. The nature and population of soil microorganisms varies depends upon the physicochemical nature of the soil like texture, pH and moisture content. Soil contains various groups of microorganisms including bacteria, fungi, yeasts and moulds (Table-2). Fourteen isolates of soil bacteria out of thirty six isolates were showed bioactivity ie) antibacterial against standard strains and clinical isolates (Table-3). LL-E 19085α, a novel antibiotic obtained from soil isolate Micromonospora citrea, potent against a spectrum of gram positive aerobic and anaerobic bacteria.[6] Micromonospora sp., SCC1792 strain was isolated from a South African soil could produce a new antifungal compound Sch 37137 is structurally related to A19009, a dipeptide isolated from a *Streptomyces sp.[*7] Resorthiomycin, a novel antitumor antibiotic was isolated from the fermentation broth of a strain of Streptomyces collinus by ethyl acetate extraction.[8] Similarly varieties of antibiotics are produced by microorganisms isolated from soil to fulfill the need for newer antibiotics to act against drug resistant developed pathogens.

Table-1 Physicochemical nature of the soil samples

S.No.	Soil Code	Color	Texture	pH	Moisture content (%)
1	S1	Dark Brown	Fine powder	7.8	0.8
2	S2	Grey	Fine powder	6.7	0.6
3	S3	Blockish Brown	Granular	7.2	1.2

Table-2 Microbiological nature of the soil samples

S.No.	Name of the site	Nature of the soil	Soil Code	No. of isolates Tested (%)	No. of Bioactive isolates (%)	Presence of biological organisms				
						B	F	A	N	W
1	Market place	Fine powder	S 1	11 (30.5%)	04 (36.4%)	Y	Y	Y	Y	Y
2	Distillery Effluents	Fine powder	S 2	11 (30.5%)	04 (36.4%)	Y	Y	Y	Y	Y
3	Garden	Granular	S 3	14 (38.9%)	06 (42.8%)	Y	Y	Y	Y	Y
			Total	36 (100%)	14 (38.9%)					

B-Bacteria, F-Fungi, A-Algae, N-Nematodes, W-Worms Y-Yes

Table - 3 Anti bacterial activity of the soil isolates against Clinical Pathogens

Soil samples	No.of soil Isolates (%)	Anti microbial activity against													
		S. aureus		*S. feacalis*		*E. coli*		*K. aerogenes*		*P. vulgaris*		*P. aureginosa*		*S. typhi*	
		+ Ve (%)	- Ve (%)	+ Ve (%)	- Ve (%)	+ Ve (%)	- Ve (%)	+ Ve (%)	- Ve (%)	+ Ve (%)	- Ve (%)	+ Ve (%)	- Ve (%)	+ Ve (%)	- Ve (%)
S1	11 (30.5%)	02 (18.2)	09 (81.8)	02 (18.2)	09 (81.8)	02 (18.2)	09 (81.8)	02 (18.2)	09 (81.8)	01 (9.1)	10 (90.9)	01 (9.1)	10 (90.9)	03 (27.3)	08 (72.7)
S2	11 (30.5%)	01 (9.1)	10 (90.9)	--	11 (100)	01 (9.1)	10 (90.9)	--	11 (100)	--	11 (100)	--	11 (100)	01 (9.1)	10 (90.9)
S3	14 (38.9)	04 (28.6)	10 (71.4)	04 (28.6)	10 (71.4)	04 (28.6)	10 (71.4)	04 (28.6)	10 (71.4)	05 (35.7)	09 (64.3)	03 (21.4)	11 (78.6)	05 (35.7)	09 (64.3)
Total	36 (100)	07 (19.4)	29 (80.5)	06 (16.7)	30 (83.3)	07 (19.4)	29 (80.5)	06 (16.7)	30 (83.3)	06 (16.7)	30 (83.3)	04 (11.1)	32 (88.8)	09 (25.0)	27 (75.0)

CONCLUSION

This study leads to the conclusion that the bacterial isolates of the study produced bioactive compounds inhibitory to the standard and clinical strains used in the screening process. This study also revealed that soil is an alternative source for microorganisms to produce novel antibiotics to overcome the problem of development of multi drug resistance of pathogenic microorganisms.

ACKNOWLEDGEMENT

The authors thank Kings Institute of Preventive Medicine, Guindy, Chennai for providing clinical strains and National Chemical Laboratory, Pune for providing standard strains.

REFERENCES

[1] Makoto mastsumoto, et.al., (1989), Isolation and Charaterisation of Thioxamycin. *J. Antibiotics* XLII (10): 1465 - 1469.

[2] Hiroo Kusakabe and Kiyoshi Isono, (1988), Taxonomic Studies on *Kitasatosporia cystarginea sp*. which produces a new antifungal antibiotic Cystargin, *J. Antibiotics* XLI (12): 1758 - 1762.

[3] Yoshinori yamashtta et.al., (2001), Chrolactomycin, a novel Antitumor Antibiotic Produced by *Streptomyces sp. J. Antibiotics* 54 (10): 836 - 839.

[4] Cappuccino, J.G. and Sherman, N., (2004), 6th edition. Microbiology A Laboratory Manual, Pearson Education (Singapore) Pvt. Ltd., Indian Branch, 482 F.I.E. Patparganj, Delhi., pp. 339-341.

[5] Bhattacharyya, R.N., (1986), Experiments with Microorganisms, 1st edition. Reprint 1993, Emkay Publications, Delhi, pp. 285-287.

[6] Maiese,W.M., et.al., (1989), LL-E 19085α, a novel Antibiotic form *Micromonospora citrea*: Taxonomy, Fermentation and Biological Activity. *J. Antibiotics* XLII (6): 846 - 851.

[7] Raymond Cooper, et al., (1988), Sch 37137, a Novel antifungal compound produced by a *Micromonospora sp*. Taxonomy, Fermentation, Isolation Structure and Biological properties. *J. Antibiotics* XLI (1): 13 - 19.

[8] Hideg Suzuki, et al., (1990), Resorthiomycin, A Novel Antitumor Antibiotic, Taxonomy, Isolation and Biological activity. *J. Antibiotics* XLIII (2): 129 - 13

.

****