International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 12 (2014) pp. 563-569**
http://www.ijcmas.com

Original Research Article

Isolation of antimicrobial producing bacteria from soil samples collected from Bhopal Region of Madhya Pradesh, India

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Keywords

Antimicrobial agent producing bacteria, Madhya Pradesh, *Pseudomonas spp*

A B S T R A C T

In the present study, a trial was done to find out a new antimicrobial agent producing bacteria from soil samples collected from different regions of Bhopal, Madhya Pradesh (India). Isolation of different bacterial colonies from soil sample was carried out. All the isolated bacterial colonies were then screened for their antimicrobial activity against the pathogenic bacteria *Salmonella typhi (MPCST-109), Serratia ficaris(MPCST-076), Streptococcus facalis (MPCST-072), Pseudomonas vesicularis (MPCST-088), Staphylococcus cohni (MPCST-121) Ecoli and Pseudomonas aeroginosa* . Among the total 28 bacterial isolates, only 12 of them (42.85%) were capable of biosynthesizing antimicrobial metabolites. One of the Bacterial colony that was obtained from Mandideep region of Bhopal, found to exhibit the highest antimicrobial activity against most of the used pathogenic bacteria in studies. The Physiochemical and biochemical characters of the isolated bacteria were matched with *Pseudomonas sps*. Thus, it was given the suggested name PBR-11. This study indicates that microorganisms isolated from Bhopal region (India) soil could be an interesting source of antimicrobial bioactive substances. Soil samples obtained from different locations of Bhopal and Guna (India) were analyzed to determine the presence and types of antibiotic-producing bacteria, using nutrient agar media.

Introduction

The problem of resistance against the present antibiotics in bacteria increases day by day. So there is an urgent need to search new antibiotics or the sources of new antibiotics. A lot of work has been done during last few decades, that has witnessed the production of novel antibiotics from different microorganisms. Soil is a primary source of microorganisms. Soil bacteria and fungi have played a significant and an important role in antibiotic discovery.

The numbers and species of microbes in soil is depend on environmental conditions like nutrient availability, soil texture, presence of moisture in soil and type of vegetation cover, and their number varies according to the type of environmental condition (Atlas and Bartha, 1998). From ancient times is well understood that, natural products have a key role in the discovery and development of many antibiotics (Newman and Cragg, 2007). One of the best approach to the

discovery of new antimicrobial agents from natural sources has been to use folklore or historical records to guide the collection of samples or a good research work on the soil of that area (Cordell *et al.*, 1994).

Antibiotics are one of the important pillars of modern medicines (Ball *et al.*, 2004), but old antibiotics lose their efficacy and they are necessarily replaced with new ones for many species of pathogenic bacteria (Hancock, 2007). Microorganisms that are able to producing secondary metabolites have a diverse chemical structure and biological activities and are produced only by some species of a genus *Bacillus* (Stachelhaus *et al*.,1995). Some of the important examples of these antibiotics used in medical treatments are bacitracin, Gramycidin S, polymyxin, and tyrotricidin (Drablos *et al*.,1999) produced by different *Bacillus spp.*

Considerable research is being done in order to find new antimicrobial producing bacteria isolated from soil (Rondon *et al.*, 2000; Crowe and Olsson, 2001; Courtis *et al.*, 2003). The present paper focus on the isolation of a bacterial strain having antimicrobial activity from soil samples collected from Bhopal region of Madhya Pradesh (India). The identification of this strain and the study of the influence of the physical and chemical conditions on the culture medium upon biosynthesis of bioactive molecules are reported

Materials and Methods

Isolation of antimicrobial agent producing microbes

Nutrient agar medium was used for the isolation of bacterial colonies from soil samples.

Isolation methods

Soil samples were collected from different localities of Bhopal region in India. Each 1 g of the sample was suspended in 9 ml sterile distilled water and shaken vigorously for 2-3 min.. The soil suspension was serially diluted in sterile normal saline (0.85%) and the dilution from 10^{-3} and 10^{-10} were then plated on overlaid Nutrient agar 0.8% with seeded test organisms and incubated at 37°C for 12 to 24 hours, to screen for antagonistic bacteria. Colonies giving a clear zone of inhibition were isolated and re-streaked over a fresh media plate. Eventually selected candidate were collected from the reservoir plate and rechecked for their activity

Preparation of bacterial supernatant for studying antibacterial activity

The isolated bacterial strains were inoculated in nutrient broth media for 48 hours. The cultures were centrifuged at 6000 rpm for 10 min. Then the supernatant was collected and stored at -70^{o}C until used. This supernatant was used to study antibacterial activity of isolated bacteria among Pathogenic bacterial species.

Test Bacteria

The test bacteria used in this study were obtained from Madhya Pradesh Council Of Science and Technology, Bhopal. They include *Salmonella typhi (MPCST-109), Serratia ficaris(MPCST-076), Streptococcus facalis (MPCST-072), Pseudomonas vesicularis (MPCST-088), Staphylococcus cohni (MPCST-121) Ecoli and Pseudomonas aeroginosa* .

Confirmation of antibacterial activity

This is the most authenticated and widely used method for studying antimicrobial

activity. Duplicate plates were used for each target organism. 100 µl of bacterial culture supernatant were added to the wells in the plates. The detection of clear inhibition zones around the wells on the inoculated plates is an indication of antimicrobial activities.

Effects of heat and enzymes on antimicrobial activity

Each bacterial supernatant were treated with 2 mg ml^{-1} trypsin (Hi-Media) at 37ºC for 1 h. For the inactivation of enzymes each samples were boiled for 2 min. Thermal stability samples were studied by exposing the supernatants at different temperatures ranging 40 to 100ºC for 15 min and 121ºC/105 kPa for 15 min before being tested for antimicrobial activity. TCA (Hi Media) was added to the filtrates so that we get a working concentration of 100 mg ml^{-1}, then the samples were incubated for 2 h at 4ºC. Samples were then centrifuged at 10,000 x *g* for 5 min and the supernatant was neutralized to pH 7.0, before testing for antimicrobial activity. After centrifugation the samples were tested for antimicrobial activity against target bacteria.

Result and Discussion

The increasing frequency of multi-resistant pathogenic bacteria is created an urgent demand in the modern world for more rational approaches and strategies to the screening of new antibiotics with a broad spectrum of activity, that can resist the inactivation processes exploited by microbial enzymes (Saadoun and Gharaibeh, 2003; Motta *et al*., 2004). This study has demonstrated that production of antimicrobial substances is widespread among the isolated bacterial strains. Screening and isolation of promising bacteria with potential antimicrobial agent is still a thrust area of research. It is necessary to search new microbes and novel metabolites and is urgent to counter the threats posed by the fast emerging phenomenon of antibiotic resistance.

Thus, in the present work, different bacterial *spp.* were isolated from soil samples collected from Bhopal region of Madhya Pradesh and then the isolated bacterial *spp*. were screened for their potential to generate antimicrobial substance. An agar well diffusion method was used to assess the production of antimicrobial compounds by bacteria isolated from soil samples against 7 pathogenic strains of bacteria. Out of 28 isolates tested, 12 (42.87%) isolates were found to exhibit antibacterial activity against pathogenic strains of bacteria.

As shown in Table- 1, the spectra of inhibition were vary among the isolates bacteria. Isolate PBR-11 showed the largest antimicrobial spectrum, exhibiting inhibitory activity against 6 pathogens, *Pseudomonas aerogenosa*, *Salmonella typhi (*MPCST-109*), E.coli*, *Pseudomonas vesicularis (*MPCST-*088), Serratia ficaris* (MPCST-076), and *Streptococcus facalis* (MPCST-072) and followed by isolate PBR-10 that inhibited 3 pathogens. The highest zone of inhibition was shown by PBRI-4 against *Pseudomonas vesicularis.* PBRI-1 also shows a greater zone of inhibition against Pseudomonas auerogenosa as shown in Table no-1.

To proved that the inhibition was due to the action of metabolite, but not due to the presence of organic acids, the supernatants were neutralized to pH 7.0 before the assay. The pH values of the crude antimicrobial substances indicate that the inhibitory effect was not due to production of organic acids.

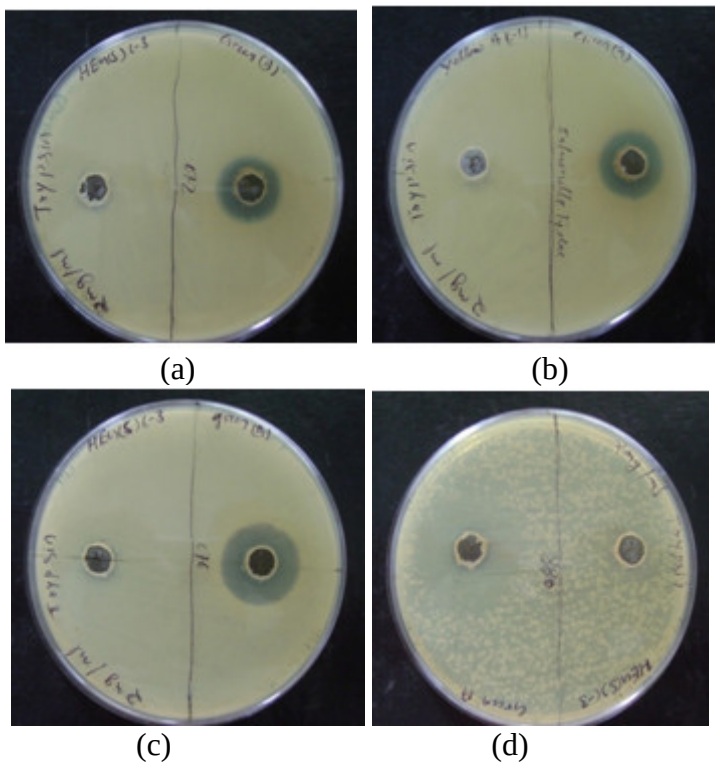
Table no.1 Antibacterial activity of isolated bacterial strains against different pathogenic strains of bacteria

Name of reference strain	**NAME OF COLONIES (Zone of inhibiton (mm)±S.D)**											
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Staphylo coccus ficaris	0	0	0	0	0	0	0	0	0	17.05±0.12	21.55±0.20	0
serratia ficcaria	0	0	11±0.32	0	12.27±0.22	0	0	0	0	17.17±0.29	24.52±0.05	0
Staphylo coccus cohni (121)	0	0	11.82±0.23	0	13.15±0.15	0	0	0	0	0	0	0
Pseudomonas vesicularis	0	26.1±0.52	0	43.52±0.25	0	0	0	0	0	13.52±0.17	34.05±0.09	0
E.coli	0	0	0	0	0	0	0	17.37±0.26	0	30.17±0.23	22.45±0.12	0
Pseudomonas aeroginosa	36.52±0.35	26.77±0.38	10.17±0.16	31.2±0.21	12.07±0.11	16.57±0.17	0	0	30.5±0.36	0	20.52±0.17	30.2±0.21
Salmonella typhy ae	0	0	0	0	12.05±0.07	18.175±0.08	15.52±0.17	0	0	16.5±0.08	24.25±0.26	28.57±0.17

Fig.1 Antagonistic effect of bacterial suspension on different pathogenic bacteria: (a) *Salmonella typhi* (b) *Streptococcus fecalis*

(a) (b)

Fig.2 Antagonistic effect of bacterial suspension after treatment with trypsin against *(a)Streptococcus fecalis (b) Salmonalle typhi*
(c) Serratia ficaris (d)Pseudomonas vesicularis.



(a) (b)

(c) (d)

Most of these substances were partially or completely inactivated by proteases and TCA, suggesting that a protein moiety is involved in the activity. This may indicate that the inhibition was due to the presence of bacteriocin-like substances. The antimicrobial substances showed high thermal resistance and low molecular weight, which are characteristics of small hydrophobic peptides that constitute class II bacteriocins (Riley and Wertz, 2002).

Although the identification of these isolated bacterial colonies were not yet done up to the *species* level, but their morphological and biochemical characteristics indicate that they belong to the genus *Bacillus* and *coccus*. Different antimicrobial compounds are produced by members of the genus *Bacillus*, most of these identified as peptides, lipopeptides and phenolic derivatives (Nakano and Zuber.,1990). Different antimicrobial substances produced by Bacillus *spp*. isolated from arthropods were recently described, including aromatic acids, acetylamino acids (amino acid analogs), and peptides (Gebhardt *et al*., 2002). Studies indicated that bacteriocin-like substances have been related to Bacillus *spp*. isolated from soil (Oscariz *et al*.,1999; Bizani and Brandelli.,2002) . Although several antimicrobial substances described in this work appear to be peptides, other substances cannot be ruled out since resistance to proteases and even to TCA was observed in some cases.

Results of this study indicate that the potential of these bacteria to produce antimicrobial compounds that can be useful for many applications is great and must be better explore. The identity of bioactive compound produced by the bacterial *spp.* is still unknown. Further analysis by protein electrophoresis and MS/MS mass spectrometry may help to reveal the identity and structure of the protein.

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