Study for Antagonistic effect of Rhizobacteria Isolated from Saline Soil of Coastal Odisha

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ABSTRACT

Eight morphologically different bacterial strains were isolated from rhizosphere saline soil of coastal Odisha, India. Among them only 3 rhizobacterial strains namead as P1, B1 and B2 belong to genus *Bacillus* had shown antibacterial activity against 5 human pathogenic bacteria in agar well diffusion method. Zone of inhibition showed by P1 against *Salmonella typhi* and *Shigella flexneri* was 8.567±0.03 and 8.133±0.03 respectively where as B2 showed 20.067±0.38 against *Streptococcus pyogenes*. B1 showed antibacterial activity against all the tested pathogens *Streptococcus pyogenes* (Gr +ve), *Salmonella typhi* (Gr -ve), *Shigella flexneri* (Gr -ve), *Streptococcus mutans* (Gr +ve), and *Vibrio cholera* (Gr -ve) i.e., 14.1±0.36, 11.767±0.21, 21.1±0.36, 21.333±0.29 and 12.1±0.2 respectively. After treatment with trypsin, organic solvents (acetone, methanol, ethanol and chloroform), heat and autoclave the metabolic activity of active components produced by test organisms were not changed.

Key words: rhizosphere, agar well diffusion, zone of inhibition, antibacterial activity and pathogen.

1. INTRODUCTION

During the last century, antibiotics produced by microorganisms have played a significant role in the treatment of infectious diseases [1]. But the problem arises with the increased resistance of microorganisms against antibiotics because of their frequent clinical use [1,2]. Microorganisms produce a variety of compounds which express antimicrobial properties. Among them, the bacteriocin is one group of compounds, which consists of relatively small bactericidal peptides . In a variety of ecological niches, microorganisms compete with each other for survival and through evolution form unique flora. These organisms are able to produce antimicrobial compounds against competing flora, including food-borne spoilage and pathogenic bacteria [3]. Soil is the primary habitat for microorganisms. Soil microorganisms have played a significant role in antibiotic discovery [2]. Considerable research is being done in order to find out soil bacteria producing new antimicrobial components [4-6]. Therefore, aim of this investigation is to find out antagonistic effect of rhizobacteria against human pathogenic organisms.

2. MATERIALS METHOD

2.1. ISOLATION OF RHIZOBACTERIA

Rhizobacteria were isolated from the saline soil collected from the rhizosphere zone of Casuarina plants from coast-line of Puri, Baleshwar and Ganjam districts of Odisha, India. Stock solution was prepared by suspending 1 gram of soil sample in 9ml of sterile distilled water and incubated on rotary shaker at 120 rpm for 10 min. 1ml of sample was serially diluted upto 10^{-10} [7]. 0.1 ml of diluted sample from each dilution tube was spreaded on sterile nutrient agar plates (0.5% peptone, 0.3% beef extract and 1.8% agar) in triplicate. The cultured plates were incubated at 37^{o}C for 72 hrs. Single colonies were picked up and streaked on sterilized nutrient agar plates to get pure culture. Well isolated healthy colonies were observed for morphological characterization. All the experiments were carried out in triplicates.

2.2. ANTIBACTERIAL ACTIVITY

Agar well diffusion method is the most authenticated and widely used method for studying antimicrobial activity [2]. The human pathogenic bacterial strains against which antibacterial activity has to be studied and all the isolated rhizobacterial strains were inoculated to nutrient broth media separately before study. After 24 hrs of incubation, the broth having rhizobacterial strains were centrifuged at 10,000 rpm for 10 minutes and supernatants were used for antimicrobial study against human pathogens; 2 Gram positive bacteria: *Streptococcus pyogenes* (MTCC 1926) and *Streptococcus mutans* (MTCC 497) *Salmonella typhi* (MTCC 1252), *Shigella flexneri* (MTCC 1457), and *Vibrio cholera* (MTCC 3907). The CFU of the test organisms 1.5×10^{8} was standardised by McFarland method (McFarland, 1907). The pathogenic strains were swabbed on the nutrient agar plates by using sterilised swabs. The well was made with help of sterile loop of diameter 5mm. About 50 µl of supernatants were loaded per well. The plates were incubated for 24 hours at 36.9^{o}C. After incubation the diameter of inhibition zone was measured. All observations were done in triplicates.

2.3. EFFECT OF TRYPSIN ON ANTIBACTERIAL ACTIVITY

To 1 ml of culture supernatant 10mg trypsin gel (Sigma, St Louis, USA) was added and incubated at 30^{o}C for 1hr on a rotary shaker. After incubation the culture supernatant was centrifuged at 15,000Xg for 5 min to remove the enzyme [8]. Antibacterial activity was assayed by agar diffusion method as described above. All the experiments were done in triplicates.

2.4. EFFECT OF ORGANIC SOLVENTS ON THE SECRETED ANTIMICROBIAL ACTIVITY

To 1 ml of culture supernatant 0.1 ml of each organic solvent (acetone, methanol, ethanol and chloroform) was added and incubated for 4 hrs at 37^{o}C [9].

2.5. EFFECT OF TEMPERATURE ON THE SECRETED ANTIMICROBIAL ACTIVITY

For heat treatment, the culture supernatants were also boiled at 100^{o}C in water bath for 1 hr, autoclaved at 121^{o}C for 10 mins and stored at -20^{o}C [9].

3. RESULT

In the present work, morphologically different bacterial strains were isolated from rhizospheric saline soil of coastal Odisha, India and tested for their antagonistic activity by agar well diffusion method (production of clear zone around the well) against 5 human pathogenic bacterial strains. Out of 8 isolated strains, 3 strains exhibited antibacterial activity against pathogenic strains which is given in table 1 and figure.2.

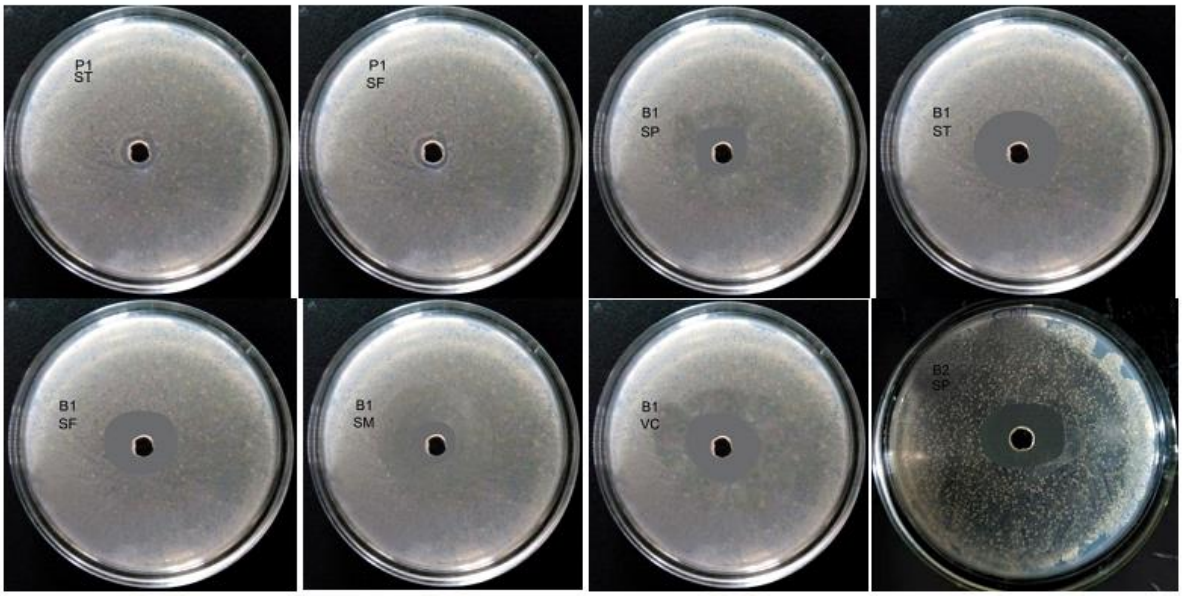

Figure 1: Isolated rhizobacterial strains showing antagonistic effect against human pathogenic bacteria.

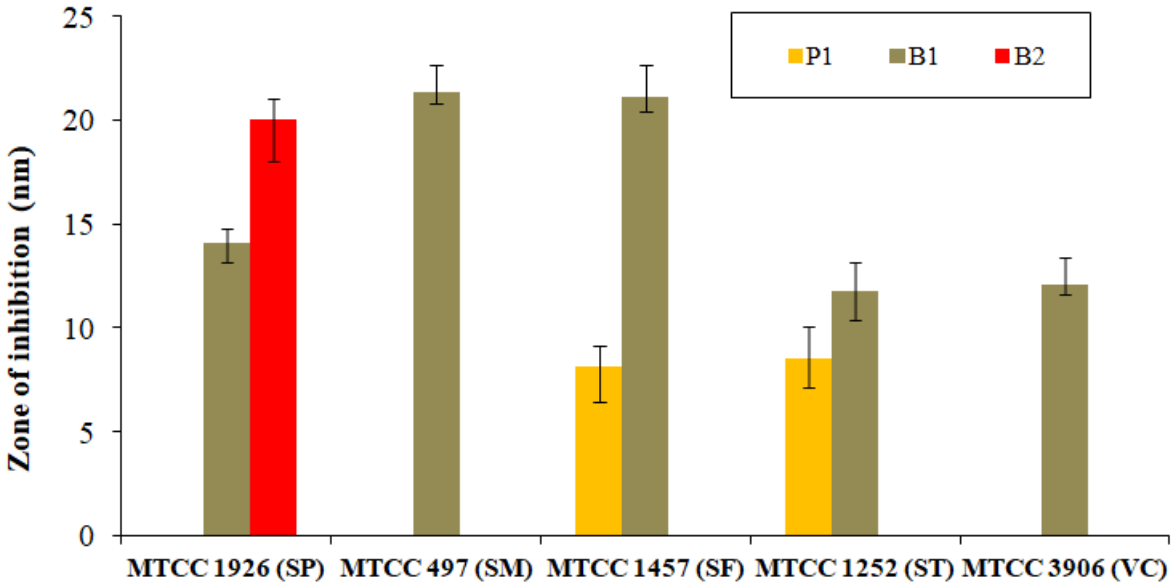

Figure.2. Antibacterial activity of isolated bacterial strains against different pathogenic strains of bacteria

Table 1: Antibacterial activity of isolated bacterial strains against different pathogenic strains of bacteria

Human pathogenic strains	Zone of inhibition by rhizobacteria*		
	P1	B1	B2
MTCC 1926 (SP)	-	14.1±0.36	20.067±0.38
MTCC 497 (SM)	-	21.333±0.29	-
MTCC 1457 (SF)	8.133±0.03	21.1±0.36	-
MTCC 1252 (ST)	8.567±0.03	11.767±0.21	-
MTCC 3906 (VC)	-	12.1±0.2	-

*= Mean of inhibition zone ± standard deviation.

Strain B1 has shown antibacterial activity against 5 pathogenic organisms whereas P1 and B2 against *Shigella flexneri*, *Salmonella typhi* and *Streptococcus pyogenes* respectively. Thus the culture supernatants of P1, B1and B2 were taken for further treatment with trypsin, organic solvents and heat.

P1, B1 and B2 were treated with trypsin to confirm the proteinaceous nature of the active components. For instance, the antibiotics produced by strains were not inactivated by trypsin (results are given in table 2) and might be nonproteinaceous in nature.

Table 2: Effect of trypsin on antibacterial activity of P1, B1 and B2

Pathogenic strains	Rhizobacteria		
	P1	B1	B2
MTCC 1926 (SP)	-	+	+
MTCC 1252 (ST)	+	+	-
MTCC 1457 (SF)	+	+	-
MTCC 497 (SM)	-	+	-
MTCC 3906 (VC)	-	+	-

+, presence of activity; -, absence of activity.

These culture supernatants of P1, B1 and B2 were also shown highly stable antagonistic effect against pathogens after treatment with organic solvents.

The antibacterial activity of the culture supernatants was not lost after 1hr treatment at 100^{o}C, 10 mins at 121^{o}C and 4 months of storage at -20^{o}C.

4. DISCUSSION

The antimicrobial substance found to be inhibitory to a wide spectrum of bacteria was reproducibly demonstrable to a high level of antagonistic potential. In the present scenario of world, it is important to discover new classes of antibiotics with a broad spectrum of activity due to increased incidence of multiple resistances exploited by microbial enzymes among pathogenic microorganisms to drugs that are currently

in clinical use [10,11]. Therefore, antagonistic activities of rhizobacteria of saline soil were investigated in this study. Total 3 isolates (25%) out of 8 isolates had exhibited antagonistic activities on agar well diffusion method. Similar result was found by Ivanova et al., 1998. They had reported that out of 491 tested marine microorganisms collected at the Pacific institute of Bioorganic Chemistry of the Far Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia, 126 strains (26%) shown antagonistic effect against pathogens. Mashoria et al., (2014) also found that out of 28 strains isolated from soil sample of Bhopal, 12 (42.87%) had shown antibacterial activity against pathogenic bacteria.

Though the isolates were not identified in species level their gram staining reactions revealed that P1, B1 and B2 strains are gram -ve, *Bacillus* sp. Perez et al., 1992, Perez et al., 1993 reported that *B. subtilis* MIR 15 strain displayed antimicrobial activity against *P. aeruginosa*, *E. coli* and *M. luteus.* Aslim et al. (2002) found that four strains belonging *B. thuringiensis*, *B. subtilis* and *B. megaterium* were active against *E. coli* and *Y. enterocolitic*a [12-14]. Oscariz et al., (1999) isolated and identified a bacteriocin producing strain of *B. cereus* from a soil sample. The strain was active against most Gram positive but not Gram-negative bacteria [15]. In the present study *Bacillus sp* (P1) has shown antibacterial effects particularly against the Gram negative bacteria whereas *Bacillus sp* (B2) has shown antibacterial activity against Gram positive test bacteria. However, Yilmaz et al., (2006) have reported that *B. cereus* M15 has inhibitory affect against both Gram positive and Gram negative bacteria [16]. The findings of this study is also indicated that *Bacillus sp* (B1) has antibacterial effect against both Gram positive and Gram negative bacteria. Ramachandran et al., (2014) also supported that B. subtilis RLID 12.1 exhibited strong and broad-spectrum antimicrobial activity towards the gram negative bacteria *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Acinetobacter baumannii*, and *Yersinia aldovae* . The antimicrobial activity was observed against the gram positive bacteria like *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Enterococcus faecalis* [17].

After treatment with trypsin, organic solvents (acetone, ethanol, methanol and chloroform) the inhibitory activities of test organisms were not decreased against the above said 5 pathogenic organisms. Von der Weid et al., 2003 also reported similar result in their study that test organisms didn't show any change in inhibition after treatment with proteolytic enzymes (proteinase K, pronase E and trypsin), organic solvents (acetone, ethanol, methanol and chloroform) [9]. Different antimicrobial compounds are produced by members of the genus *Bacillus*, most of these identified as peptides, lipopeptides and phenolic derivatives [18]. Ramachandran et al., (2014) also reported that B. subtilis RLID 12.1 didn't lose any antibacterial activity after treatment with trypsin and organic solvents[17].

The antimicrobial substance was found to be heat stable at all temperatures tested. Ramachandran et al., (2014) also supported that B. subtilis RLID 12.1, retained 100% activity at 37°C for 5 h, 95% and 88% at 50°C and 60°C for 3 h respectively, 85% at 70°C and 80°C for 2 h and 1 h respectively, 82% at 90°C and 100°C for 1 h respectively, and 72% activity at 121°C for 40 min [17].

Also no changes in inhibition zone were found after treatment with heat and storage at -20ºC. Von der Weid et al., 2003 also reported similar result that antimicrobial activity of the 10-fold-concentrated supernatant was found to be stable for at least 4 months at -20ºC and the activity of the 10 fold concentrated supernatant was not lost after 1 h at 100ºC or after autoclaved at 121ºC for 10 min.

5. CONCLUSION

The present investigation reveals the broad-spectrum antimicrobial potential of the wild type isolates P1, B1 and B2 identified as *Bacillus sp*. It produces highly heat stable substances. The present characterization revealed interesting properties which justifies its potential application in the biological control of pathogenic strains.This work presents an opportunity to develop novel therapeutic agents against common bacterial infections that will benefit the vulnerable sections of the society.

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