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Research Article

Evaluation of Antimicrobial Activity of *Bacillus cereus* And *Bacillus pumillus* Metaboites Against Human Pathogens.

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ABSTRACT

Soil samples were screened for bacteria with antibiotic production potential against human pathogens including bacteria and fungi. Crude metabolites of the selected 12 isolates were tested for antibacterial activity. The best two isolates were selected for further antimicrobial screening. The two bacteria were identified as *Bacillus cereus* and *Bacillus pumilus*. The metabolites of the two bacteria were subjected to various solvent extractions and the extracts were tested for antibacterial and antifungal activity. The ethyl acetate extracts of both the organisms showed good antibacterial activity but insignificant antifungal activity. The Minimum Inhibitory Concentration (MIC) studies proved the potency of the isolates for antibiotic production. During TLC-bioautographic studies, the bands obtained at Rf values 0.87 and 0.85 showed components with antibacterial properties for *Bacillus cereus* and *Bacillus pumilus* respectively.

Key words: *Bacillus cereus, Bacillus pumilus,* antimicrobial activity, solvent extraction, human pathogens.

INTRODUCTION

Microorganisms are known to produce some of the most important medicines for various diseases. They are the source of many life saving drugs and also effective antibiotics against bacterial and fungal infections [1]. After the discovery of penicillin in 1928, antibiotics have been recognized as the only means of effective control of microorganisms. Since then, there has been continuous search for

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more effective antibiotics [2, 3, 4]. In spite of tremendous success of secondary metabolite research for antibiotics, the numbers of antibiotics are currently approaching a saturation curve with an apparent limit of application in the near future. Along with the usage of new antibiotics as therapeutics, there is emerging menace of drug resistance among microorganisms worldwide. The increase in antibiotic resistance has been attributed to inappropriate usage and inadequacies on the part of the manufacturers, thereby steady decline of effective antibiotics [5, 6]. Due to the above said facts today, there is increasing demand for new lead molecules as antimicrobials and has enforced to search for novel organisms with new metabolites in so far untouched habitats[1]. Soil is an intensively exploited ecological niche for inhabitants to produce many biologically active natural products, including clinically important antibiotics. About 75-80% of the commercially and medically useful antibiotics have been derived from bacteria especially from the genus *streptomyces* sp. obtained from soil [7]. Till recently many new small molecular drugs of microbial origin isolated from soil were approved by Food and Drug Association (FDA) in the antibacterial area [8]. This proves microbes have still remained as constant and eternal source of new antimicrobial agents overcoming new snags and challenges.

Our interest was focused on screening the soil samples for bacteria with antibiotic production potential from Malnad area of Shimoga district, Karnataka, India. This is a part of western ghat region. Malnad area of Shimoga district is less exploited for this purpose. With a humid tropical climate harboring rivers, and rain forests, the microbial strains that exist in this area with its diversity may provide rare and novel antibiotics.

MATERIALS AND METHODS

Collection of soil samples and isolation of antibiotic producing bacteria: Soil samples were collected from a hill station nearby Agumbe forest region, Shimoga district, Karnataka. The organisms present in the soil were screened for their antibiotic production potential by crowded plate technique [9]. Nutrient agar was used for bacterial isolation in place of Tripticase soy agar media. Twelve bacteria that showed promising activity were selected, isolated in pure form and preserved at 4^{o}C for further screening.

Identification of bacteria: Morphological and biochemical tests were carried for the two bacteria KBAIA-0210 and JKNAB-0609. The lipid profile was also performed. The two bacteria were identified as *B. cereus* and *B. pumilus* respectively.

Testing of metabolites for antibacterial activity by agar well diffusion method: The preserved bacteria were grown in nutrient broth medium incubated at 35^{o}C for three days. After three days the broth was centrifuged at 10,000 x g for 20 minutes to remove the cells. The supernatant containing

the active metabolites was collected. The crude metabolite of all the bacteria were tested for their antibacterial activity against two gram positive bacteria (*Staphylococcus aureus* MTCC 3160 and *Streptococcus pyogens* MTCC 7028) and two gram negative bacteria (*Escherichia coli* MTCC 723 and *Pseudomonas aeruginosa* MTCC 3541) by agar well diffusion method [10]. The metabolites of two bacteria (KBAIA-0210 and JKNAB-0609) which showed maximum zone of inhibition against the target bacteria were selected for further antimicrobial study.

Solvent extraction and preparation of samples: The two bacteria, *B. cereus* and *B. pumilus* were grown separately in large quantity nutrient broth medium and incubated for three days at 35^{o}C. The broth was centrifuged to separate the cells at 10,000 x g for 20 minutes. The clear supernatant containing the metabolites was collected. The metabolites of both organisms were subjected to successive solvent extraction with petroleum ether, ethyl acetate and methanol (1:1) in a separating funnel. All the three solvent extracts were dried in separate plates. The *B. cereus* (BC) petroleum ether extract was labeled as sample BC-1, ethyl acetate extract as BC-2 and methanol extract as BC-3. Similarly the *B. pumilus* (BP) extracts were labeled as BP-1, BP-2 and BP-3 for petroleum ether extract, ethyl acetate extract and methanol extract respectively.

Antibacterial screening: The antibacterial screening was carried by agar disc-diffusion method. The Mueller-Hinton agar (MHA) was used for disc diffusion susceptibility testing. The discs were prepared by loading 30 µg of sample per disc. The standard Ciprofloxacin disc (30 µg) was also prepared and used for comparison of the antibacterial activity. The antibacterial test was carried on seven target bacteria- *Staphylococcus aureus (MTCC 3160), Escherichia coli (MTCC 723), Klebsiella pneumoniae (MTCC 7028), Pseudomonas aeruginosa (MTCC 3541), Salmonella typhi (MTCC 734), Streptococcus pyogens (MTCC 1924) and Proteus vulgaris (MTCC 1771).*

Agar disc diffusion: The preparation of test inoculums, inoculation procedure and placement of discs were performed as per National Committee for Clinical Laboratory Standards [11]. The flow sheet of overall procedure of the disc diffusion antimicrobial susceptibility test is shown in Figure 1**.**

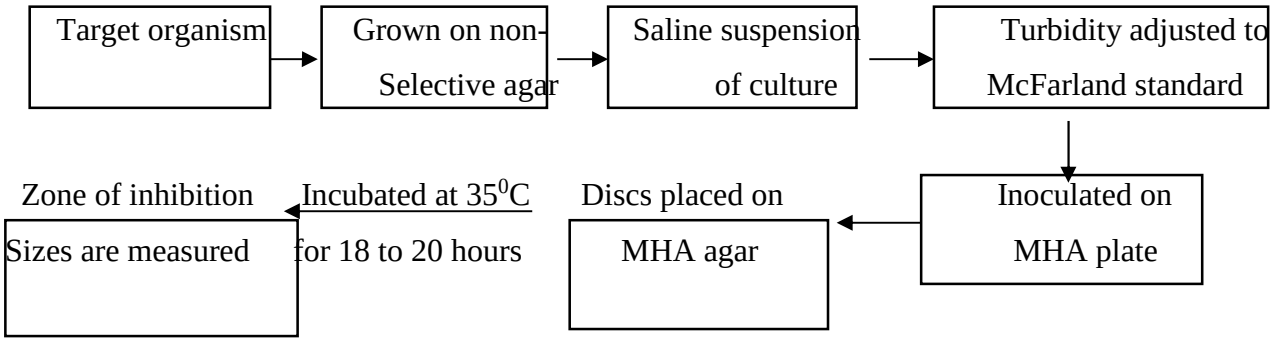

Fig 1. Flow sheet of antimicrobial susceptibility testing by disc diffusion method.

Antifungal screening: The antifungal screening was carried by disc diffusion method, similar to antibacterial screening. Antifungal tests were carried against six target human pathogenic fungi, such as *Candida albicans (MTCC 1637), Chrysosporium indicum (MTCC 4395), Chrysosporium merdarium (MTCC 4608), Chrysosporium keratinophilum (MTCC 1367) Trichophyton rubrum (MTCC 3272)* and *Microsporum gypsium (MTCC 2819).* Clotrimazole was used as standard for comparison of antifungal activity. The medium used for growth of the fungi was Sabouraud Dextrose Broth and Sabouraud Dextrose Agar. After placing the antimicrobial discs over the medium plates, the plates were incubated at 28°C for 72 hours.

Determination of minimum inhibitory concentration (MIC): The MIC was determined by modified resazurin method of antibacterial assay as previously described [12]. The MIC studies were carried for ethyl acetate extracts of *B. cereus* (BC-2) and *B. pumilus* (BP-2). The test organisms used were *E. coli, K. pneumoniae, P. aeruginosa* and *S. typhi.*

TLC-Bioautographic analysis: Thin layer chromatography (TLC) was performed for samples BC-2 and BP-2. TLC were developed on Merck TLC F254 plates with Hexane:Chloroform:Methanol (7:2:1.5) as mobile phase for BC-2 and Hexane:Chloroform:Methanol (6:2:1.5) for BP-2. The separated components were visualized under visible and UV-light at 254nm and 360 nm.

For bioautographic analysis, the developed TLC plates were dried overnight and sprayed with a concentrated suspension of actively growing *K. pneumoniae*. The plates were sprayed with 2 mg/ml solution of MTT (3-[4, 5-Diemethylthiazol – 2-yl] 2, 5-diphenyltetrazolium Bromide). The plates were incubated at 37°C in a chamber at 100% relative humidity for 18 hours and visualized [13]. The TLC fractions of BC-2 and BP-2 with Rf value 0.87 and 0.85 was subjected to GC-MS analysis and FTIR spectroscopy (Shimadzu-8300).

Statistical analysis: Data obtained from the results were statistically analyzed using SPSS 10.0 statistic software. The statistical significance was evaluated using Duncan's test and Student's t-test was used to analysis the difference between experimental group and control.

RESULTS

Agar well diffusion of metabolites: Among the 12 bacterial isolates tested for their antimicrobial activity, the crude metabolites of two bacteria KBAIA-0210 and JKNAB-0609 showed more consistent activity against all the target bacteria. They also showed good zone of inhibition against the target bacteria when compared with the other isolates. The zone of inhibition obtained for crude metabolites are shown in Figure 2. Identification of bacteria: After carrying morphological, biochemical tests and lipid profile analysis, the bacterial isolate KBAIA-0210 was identified as

Bacillus cereus and JKNAB-0609 was identified as *Bacillus pumilus*. The results of morphological and biochemical studies of KBAIA-0210 and JKNAB-0609 are shown in Table 1.

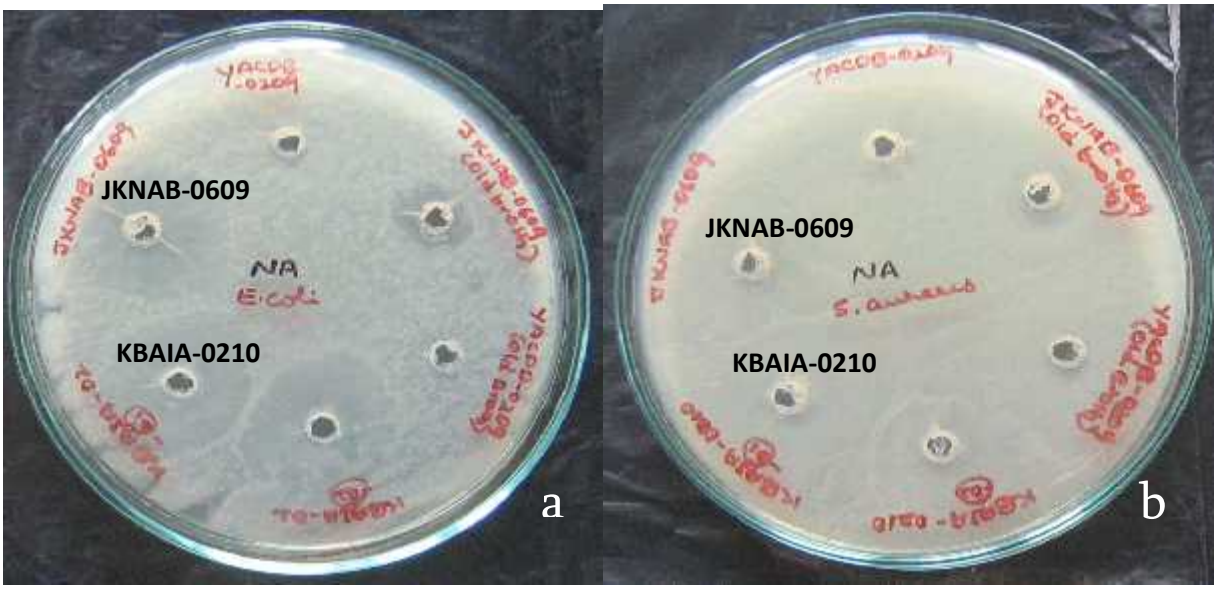

Fig. 2. Crude extracts of KBAIA-0210 and JKNAB-0609 showing zone of inhibition in well diffusion method against Escherichia coli (a) and Staphylococcus aureus (b).

Table 1. Morphological and biochemical characterization of KBAIA-0210 and JKNAB-0609 *Bacillus* strains.

Sl. no	Biochemical tests	Culture ID		Identification
		KBAIA-0210	JKNAB-0609	
01	Gram staining	Gram +ve rods	Gram +ve rods	
02	Spore staining	Positive	Positive	
03	Catalase	Positive	Positive	*Bacillus cereus*
04	Oxidase	Negative	Negative	KABALA-0210)
05	Voges-Proskauer	Positive	Positive	*Bacillus pumilus*
06	Nitrate reduction	Positive	Negative	(JKNAB-0609)
07	Growth on 7% NaCl	Positive	Positive	
08	Growth at 50^0C	Negative	Positive	
09	Growth at 65^0C	Negative	Negative	
10	Casein hydrolysis	Positive	Positive	
11	Glucose (acid)	Positive	Negative	
12	Glucose (gas)	Negative	Negative	
13	Starch hydrolysis	Positive	Negative	

Antibacterial screening by disc-diffusion: The petroleum ether, ethyl acetate and methanol extracts of *B. cereus* and *B. Pumilus* tested for their antibacterial activity showed significant but varying degree of activity on target bacteria. The sample BC-1 showed maximum zone of inhibition against *E. coli* (18 mm) and *P. vulgaris* (10 mm), the sample BC-2 showed maximum zone of inhibition against *P. aeruginosa* (20 mm) and *K. pneumoniae* (17 mm), and sample BC-3 showed maximum zone of inhibition against *P. aeruginosa* (20mm) and *S. aureus* (18 mm). Similarly, the sample BP-1 showed maximum zone of inhibition against *S. aureus* (20 mm), *P. aeruginosa* (20 mm), BP-2 showed maximum zone of inhibition against *K. pneumoniae* (20 mm), *S. aureus* (18 mm) and BP-3 showed maximum zone of inhibition against *Salmonella typhi* (15 mm) and *P. aeruginosa* (12 mm) respectively. The variations exhibited in zone of inhibition by different solvent extracts indicate that different compounds are eluted during solvent extractions and are responsible for antibacterial activity. The results obtained from disc diffusion test are shown in Table 2.

Table 2. The zone of inhibition exhibited by solvent extracts of *Bacillus cereus* and *Bacillus pumilus* against target bacteria by disc diffusion method (30 µg/disc).

	BC-1		BC-2		BC-3		BP-1		BP-2		BP-3		Cf
Strain No.	MD (mm)	IRM (%)	MD (mm)	IRM (%)	MD (mm)	IRM (%)	MD (mm)	IRM (%)	MD (mm)	IRM (%)	MD (mm)	IRM (%)	MD (mm)
1	6.0 ± 1.0	30.0[a]	14.0 ±1.9	70.0[d]	18.0* ± 2.4	90.0[b]	20.0* ±2.1	99.5[f]	18.0* ±2.7	90.4[f]	10.0 ±1.7	50.5[d]	20.0 ±2.4
2	18.0 ± 2.3	75.4[f]	12.0 ±1.5	50.0[c]	14.0* ± 1.7	58.3[d]	8.0 ±1.3	33.4[a]	8.0 ±1.3	33.4[a]	8.0 ±1.2	33.4[b]	24.0 ±
3	10.0 ± 1.9	47.3[c]	17.0* ±2.1	80.9[e]	7.0 ± 1.4	33.3[a]	7.0 ±1.1	33.4[a]	20.0* ±2.7	95.4[g]	7.0 ±1.3	33.6[b]	21.0 ±2.0
4	16.0* ± 2.1	72.4[e]	18.0* ±2.5	81.8[e]	20.0* ± 2.3	90.9[f]	20.0 ±2.3	90.4[e]	16.0* ±2.0	72.4[e]	12.0 ±1.6	54.6[e]	22.0 ±2.7
5	8.0 ± 1.3	36.3[b]	6.0* ±0.9	27.2[a]	14.0 ± 1.5	63.6[e]	8.0 ±1.0	36.2[b]	8.0 ±1.6	36.2[b]	15.0 ±1.3	68.6[f]	22.0 ±2.5
6	7.0* ± 0.80	35.6[b]	14.0* ±0.61	70.0[d]	8.0* ± 0.62	40.0[c]	12.0* ±1.15	60.4[d]	10.0* ±0.85	50.4[c]	7.0* ±0.58	35.5[c]	20.0 ±1.0
7	10.0* ± 0.95	50.4[d]	8.0* ±0.62	40.0[b]	7.0* ± 0.55	35.0[b]	8.0 ±1.0	40.4[c]	12.0* ±0.75	60.4[d]	6.0 ±0.62	30.6[a]	20.0 ±1.25

Note: [1]Strain 1 (*S. aureus*), Strain 2 (*E. coli*), Strain 3 (*K. pneumoniae*), Strain 4 (*P. aeruginosa*), Strain 5 (*S. typhi*), Strain 6 (*S. pyogens*), Strain 7 (*P. vulgaris*), [2]MD: mm Colony diameter, [3]IRM: % Inhibitory rate, [4]Mean ± standard error, [5]Mean followed * indicates level of significance at p <0.05 when compared to standard, [6]Petroleum ether extract of *B. cereus* (BC-1), [7]Ethyl acetate extract of *B. cereus* (BC-2), [8]Methanol extract of *B. cereus* (BC-3). [9]Petroleum ether extract of *Bacillus pumilus* (BP-1), [10]Ethyl acetate extract of *Bacillus pumilus* (BP-2), [11]Methanol extract of *Bacillus pumilus* (BP-3), [12]Standard Ciprofloxacin (Cf), [13]SE followed by same superscripts in a column are not significantly different where as values with different superscripts are significantly different from one another at 0.05 significance level (Duncan's test).

Figure 3 shows the zone of inhibition exhibited by microbial extracts against target pathogenic bacterial and Figure 4 shows the MIC activity of test organisms against target pathogenic bacteria.

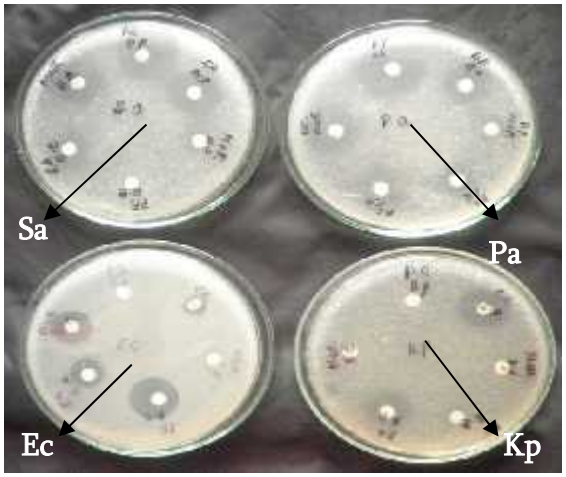

Fig. 3. Solvent extracts of Bacillus Cereus and Bacillus Pumilus showing zone of inhibition against target bacterial pathogens, Staphylococcus aureus (Sa), Pseudomonas aeruginosa (Pa), Escherichia coli (Ec) and Klebsiella pneumonia (Kp) in disc-diffusion method.

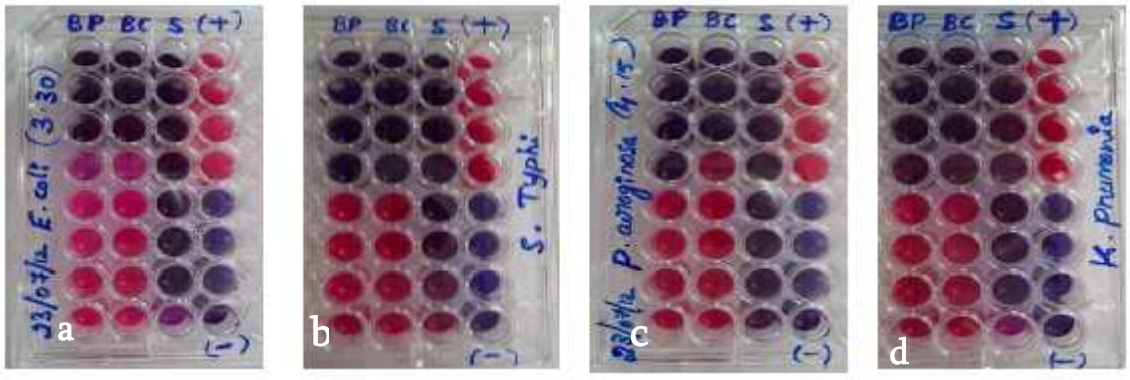

Fig. 4. Photographs showing color change from purple to pink indicating the growth. The lowest concentration at which color change do not occur is taken as MIC value of Bacillus pumulis and

Bacillus cereus against target pathogens, Escherichia coli (a), Salmonella typhi (b) , Pseudomonas aeruginosa (c), Klebsiella pneumoniae (d).

Antifungal screening: No significant activity was observed against target fungi. The results of antifungal disc diffusion are shown in Table 3.

Table 3. The zone of inhibition exhibited by solvent extracts of *Bacillus cereus* and *Bacillus pumilus* against target fungi by disc diffusion method (30 µg/disc).

	BC-1		BC-2		BC-3		BP-1		BP-2		BP-3		Cf
Strain No.	MD (mm)	IRM (%)	MD (mm)	IRM (%)	MD (mm)	IRM (%)	MD (mm)	IRM (%)	MD (mm)	IRM (%)	MD (mm)	IRM (%)	MD (mm)
1	NA	0.0	NA	0.0	6.0 ±1.1	30.0[c]	5.0 ±0.5	24.9[a]	8.0 ±2.1	40.6[d]	5.0 ±0.9	25.5[a]	20.0 ±2.4
2	5.0 ± 0.8	19.2[a]	NA	0.0	NA	0.0	8.0 ±1.3	33.4[a]	6.0 ±1.2	23.5[b]	8.0 ±1.1	30.4[b]	26.0 ±2.7
3	6.0 ± 0.9	21.8[b]	8.0 ±1.3	28.4[b]	6.0 ± 0.8	21.4[a]	8.0 ±1.2	28.5[b]	6.0 ±1.0	20.9[a]	9.0 ±1.7	1.5[b]	21.0 ±2.0
4	NA	0.0	6.0 ±1.0	20.5[a]	7 ±1.2	23.4[b]	NA	0.0	NA	0.0	8 .0 ±1.5	26.0[a]	30.0 ±3
5	6.0 ± 0.7	21.9[b]	8.0 ±2.0	28.1[b]	6.0 ± 0.7	21.7[a]	8.0 ±1.5	28.5[b]	7.0 ±1.4	24.9[c]	NA	0.0	28.0 ±2.5
6	8.0 ± 1.1	33.3[c]	7.0 ±2.1	29.5[b]	NA	0.0	6.0 ±1.0	25.5[a]	NA	0.0	NA ±0.58	0.0	24.0 ±2.1

Note: [1]Strain 1 (*C. albicans*), Strain 2 (*C. indicum*), Strain 3 (*C. merdarium*), Strain 4 (*C. keratinophilum*), Strain 5 (*T. rubrum*), Strain 6 (*M. gypsium*), [2]MD: mm Colony diameter, [3]IRM: % Inhibitory rate, [4]Mean ± standard error, [5]Mean followed * indicates level of significance at $p < 0.05$ when compared to standard, [6]Petroleum ether extract of *B. cereus* (BC-1), [7]Ethyl acetate extract of *B. cereus* (BC-2), [8]Methanol extract of *B. cereus* (BC-3). [9]Petroleum ether extract of *Bacillus pumilus* (BP-1), [10]Ethyl acetate extract of *Bacillus pumilus* (BP-2), [11]Methanol extract of *Bacillus pumilus* (BP-3), [12]Standard Ciprofloxacin (Cf), [13]SE followed by same superscripts in a column are not significantly different where as values with different superscripts are significantly different from one another at 0.05 significance level (Duncan's test).

Minimum inhibitory concentration (MIC): The BC-2 and BP-2 samples showed MIC at 125µg/ml against *S. typhi* and *K. pneumoniae*. The MIC showed against *E .coli* was at little higher

concentration of 250µg/ml for both the samples. However, the MIC showed against *P. aeruginosa* was 125µg/ml for BC-2 and 250µg/ml for BP-2. The results of MIC values are shown in Table 4.

Table 4. The MIC values of ethyl acetate extracts of *Bacillus cereus* and *Bacillus pumilus* against the target bacteria

Test organisms	MIC of Standard Ciproflaxacin (µg/ml)	MIC values (µg/ml)	
		BC-2	BP-2
E. coli	15.6	250	250
S. typhi	15.6	125	125
P. aeruginosa	7.8	125	250
K. pneumoniae	15.6	125	125

Note: [1]Minimum Inhibitory Concentration (MIC), [2]Ethyl acetate extract of *B. cereus* (BC-2), [3]Ethyl acetate extract of *B. pumilus* (BP-2).

TLC-Bioautographic analysis: The TLC mobile phase developed for BC-2 separated the compounds over a wide range of Rf values. The bands obtained at Rf value 0.87 demonstrated inhibitory components against *K. pneumoniae*. The TLC mobile phase that was developed for BP-2 demonstrated inhibitory components at Rf value 0.85 against *K. pneumoniae.*

The GC-MS analysis results indicate that inhibitory fractions of both samples BC-2 and BP-2 are not single compounds but a group of compounds with smaller molecular weights. The FTIR spectra showed the presence of Hydroxyl groups (Results not shown).

DISCUSSION

Bacillus species are known to inhabit soil and the organisms are well documented to withstand both high and low temperature conditions. Most of the *Bacillus* species are known to have potential to produce high quality antibiotics [3]. "Bacteriocin" is found to be the most common antibiotic production in many *Bacillus* species. Bacteriocins are ribosomally synthesized antimicrobial peptides [14]. The antagonistic mechanism was studied at the cell morphology and cell physiology of *Botrytis cinerea* spores and distorted hyphae using *B. cereus* strain fermentation broth. Hyphal cell configuration of *B. cinerea* was changed especially in cell nucleus, mitochondrion, and so on. This provided basis for the study on antagonistic mechanism of *B. cereus* against fungal pathogen [15].

Different species of *Bacillus* produce different bacteriocins. They include "subtilin" from *Bacillus subtilis*, "Coagulin" from *Bacillus Coagulans*, "Thuricin" from *Bacillus thuringiensis*. Similarly many strains of genus *Bacillus* are known to produce several important peptide antibiotics like Bacitracin, Polymyxin, Gramicidin, Tyrosidine and Bacilysin. The compounds like Bacillomycin,

Mycobacillin and Fungistatin obtained from *Bacillus* species are effective against molds and yeasts [16]. The species isolated in the present work belongs to the same genus *Bacillus* and were identified as *Bacillus cereus* and *Bacillus pumilus*.

Bacillus Pumilus: There are some reports of *B. Pumilus* isolates demonstrating good antibacterial activity. Antibacterial activity for crude metabolites of *B. pumilus* isolate was studies by earlier workers [17, 18]. Most of the compounds reported are peptide antibiotics [19 20, 21, 22]. "Bacitracin", a peptide antibiotic produced by *B. Pumilus* has been reported and several Bacitracins have been characterized [16, 23]. The compounds responsible for antimicrobial activity of *B. Pumilus* isolates are due to presence of lipopeptides and lipoamides [18]. The metabolites obtained in the present work were found to be non-peptides. They have also reported antibacterial activity of these metabolites against *S. aureus* (14 mm), *P. aeruginosa* (13 mm) and *P. vulgaris* (19 mm). In the present study the samples BC-2, BC-3, BP-1, and BP-2 have shown a zone of inhibition up to 20 mm against *S. aureus, P. aeruginosa* and *P. vulgaris* which is comparatively a better potency compared to earlier results. No activity of *B. pumilus* isolate against *P. aeruginosa* and *E. coli* were noted by earlier worker whereas the present isolate has demonstrated 20 mm and 8 mm zone of inhibition against the same organisms respectively [24]. 5 mm zone of inhibition against *S. aureus* and 6 mm zone of inhibition against *K. pneumoniae* were observed by earlier reporter whereas the present isolate has demonstrated 20 mm zone of inhibition against the same target bacteria [3]. Many studies on antifungal activity of *B. Pumilus* strains mainly against plant pathogens have been reported [25, 26, 27, 28]. Whereas the result of the present study has shown moderate antifungal activity against all test fungal human pathogen except *C. keratinophilum.* Metabolites of the present isolates had failed to show any significant antifungal activity when compared to control antibiotic, Clotrimazole [18].

Bacillus cereus: *B. cereus* is a spore forming bacteria that occurs naturally in many kinds of foods and produce 'emetic toxin' causing illness to humans [29]. Several strains of *B. cereus* have been reported for their antibacterial activity [24, 30, 31]. Most of the antibacterial compounds reported are peptide antibiotics like Biocerin, Cerexin and Thiocillin [16, 32]. Earlier report has shown no activity of *B. cereus* isolate obtained from soil sample in Minna city, Nigeria against *S. aureus* and *P. aeruginosa* [21]. The present isolated strain of *B. cereus* has shown 18 mm and 20 mm zone of inhibition against the same target bacteria. They also reported 12 mm zone of inhibition against *K. pneumoniae* and 14 mm zone of inhibition against *S. typhi* whereas the present isolate has demonstrated 17 mm and 14 mm zone of inhibition against same pathogens respectively. These results indicate that the present isolate is having more potent antibacterial activity than reported in earlier studies. Earlier worker reported MIC with more than 625µg/ml against *E. coli* [30]. The present isolate has shown MIC of 250µg/ml against *E. coli*. Many strains of *B. cereus* with antifungal

activity have been reported. Different strains of *B. cereus* are known to produce antifungal peptide antibiotics like Mycocerin and Cerulide [33, 34]. Some strains are known to produce antifungal peptides of Iturin group [35] and Bacereutin [36]. *B. cereus DGA 34* and *B. cereus UW 85* are known to produce Zwittermicin A, which inhibit the growth of fungal plant pathogens. The metabolites of *B. cereus B4* are used as pesticide as well as to improve crop strength [37]. In the present work, the metabolites of *B. cereus* and *B. pumilus* targeted against the human pathogens have shown significant results. Hence the metabolite of *B. cereus* and *B. pumilus* can be applicable to treat human pathogens mainly against bacterial pathogens.

CONCLUSION

The ethyl acetate fractions of the *Bacillus cereus* and *Bacillus pumilus* have shown the potential of producing antibacterial compounds when compared to antifungal compounds. The MIC studies and TLC-bioautographic studies have proved the potency of antibiotic production. The mass spectral studies of TLC fractions revealed that antibacterial activity may be due to the group of compounds of smaller molecular weights, but not peptide antibiotic. Further purification of the TLC fractions is necessary for structural analysis of the compounds. The further purification and their spectral analysis may provide a potential antibacterial lead molecule.

REFERENCES

1. Thakur D, Yadav Y, Gogoi BK and Bora TC. (2007). Isolation and screening of *Streptomyces* in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites. *J. Med. Mycol*. 17: 242-249.
2. Guy ES and Mallampalli A. (2008). Managing TB in the 21st century: existing and novel drug therapies. T*her. Adv. Respir. Dis.* 2: 401-8.
3. Kuta FA. 2008. Antifungal effects of *Calotropis procera* stem bark on *Epidermophyton flocosum* and *Trichophyton gypseum*. *Afr. J. Biotechnol.* 7: 2116-2118.
4. Song JH. 2008. What's new on the antimicrobial horizon?. *Int. J. Antimicrob. Agents.* 32: 207-13.
5. Byarugaba DK. (2004). A view on antimicrobial resistance in developing countries and responsible risk factors. *Int. J. Antimicrob. Agents*. 24:105-110.
6. Blomberg B. 2008. Antimicrobial resistance in developing countries. *Journal of the Norwegian Medical Association*. 128: 2462-2466.

7. Mellouli L, Ameur-Medhi RB, Sioud S, Salem M and Bejar S. (2003). Isolation, purification and partial characterization of antibacterial activities produced by a newly isolated *Streptomyces* sp. US24 strain. *Res. Microbiol*. 154: 345–352.
8. Sivaramkrishna H and Mahajan G. (2009). Microbes: An eternal source of innovative drugs. www.expresspharmaoline.com.
9. Cappuccino J and Sherman N. (2004). Isolation of antibiotics producing microorganisms and determination of antimicrobial spectrum of isolates (Expt No. 54), pp 333. Microbiology, A laboratory manual, Pearson Education, Inc.
10. Haque SF, Sen SK and Pal SC. (1995). Nutrient optimization for production of broad spectrum antibiotic by *Streptomyces antibioticus* SR15.4. *Acta. Microbiol. Immunol. Hung.* 42: 155-62.
11. National Committee for Clinical Laboratory Standards. (1999). Performance standards for antimicrobial susceptibility testing; Ninth informational supplement. Wayne, Pennsylvania, nccls. pp. 1526-1543.
12. Sarker SD, Nahar L and Kumarasamy Y. (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods*. 42: 321–324.
13. Begue WJ and Kline RM. (1972). The use of tetrazolium salts in bioautographic procedures. *J. Chromatogr*. 64: 182-4.
14. Pattnaik P, Grover S and Batish VK. (2005). Effect of environmental factors on production of lichenin, a chromosomally encoded bacteriocin-like compound produced by *Bacillus licheniformis* 26L-10/3RA. *Microbiol Res.* 160: 213-8.
15. Li FX, Ma HQ, Liu J and Zhang C. (2012). Antagonistic Effects of *Bacillus cereus* Strain B-02 on Morphology, Ultrastructure and Cytophysiology of *Botrytis cinerea*. *Pol. J. Microbiol.* 61: 119-128.
16. Katz E and Demain AL. (1977). The peptide antibiotics of Bacillus: chemistry, biogenesis, and possible functions. *Bacteriol. Rev*. 41: 449–474.
17. Hasan F, Khan S, Shah AA and Hameed A. 2009. Production of Antibacterial Compounds by Free and Immobilized *Bacillus Pumilus* SAF1. *Pak. J. Bot*. 41: 1499-1510.
18. Berrue F, Ibrahim A, Boland P and Kerr RG. (2009). Newly isolated marine *Bacillus pumilus* (SP21): A source of novel lipoamides and other antimicrobial agents. *Pure Appl. Chem.* 81: 1027–1031.
19. Aunpad R, Na-Bangchang K and Pumilicin. (2007). A Novel Bacteriocin with Anti-MRSA and Anti-VRE Activity Produced by Newly Isolated Bacteria *Bacillus pumilus* Strain WAPB4. *Curr. Microbiol.* 55: 308-13.

20. Awais M, Pervez A, Qayyum S and Saleem M. (2008). Effects of glucose, incubation period and pH on the production of peptide antibiotics by *Bacillus pumilus*. *African J. Microbio. Res*. 2: 114-119.
21. Quoba LII, Diawara B, Jespersen L and Jakobsen M. (2007). Antimicrobial activity of *Bacillus subtilis* and *Bacillus pumilus* during the fermentation of African locust bean (*Parkia biglobosa*) for Soumbala production. *J. Appl. Microbiol*. 102: 963–970.
22. Awais M, Shah AA, Hameed A and Hasan F. (2007). Isolation, identification and optimization of Bacitracin produced by *Bacillus* sp. *Pak. J. Bot*. 39: 1303-1312.
23. Schallmey M, Singh A and Ward OP. (2004). Developments in the use of *Bacillus* species for industrial production. *Can. J. Microbiol.* 50: 1-17.
24. Kuta FA, Mimzing L and Orka APY. (2009). Screening of *Bacillus* species with potentials of antibiotics production. *Applied Medical Informatics*. 24: 42-46.
25. Leifert C, Li H, Chidburee S, Hampson S, Workman S, Sigee D, Epton HA and Harbour A. (1995). Antibiotic production and biocontrol activity by Bacillus subtilis CL27 and *Bacillus pumilus* CL45. *J. Appl. Bacteriol.* 78: 97-108.
26. Munimbazi C and Bullerman LB. (1998). Isolation and partial characterization of antifungal metabolites of *Bacillus pumilus*. *J. Appl. Microbiol.* 84: 959-68.
27. Bernal G, Illanes A and Ciampi L. (2002). Isolation and partial purification of a metabolite from a mutant strain of *Bacillus sp*. with antibiotic activity against plant pathogenic agents. *Electronic Journal of Biotechnology.* 5: 12-20.
28. Bottone EJ and Peluso RW. (2003). Production by *Bacillus pumilus* (MSH) of an antifungal compound that is active against *Mucoraceae* and *Aspergillus* species: preliminary report. *J. Med. Microbiol.* 52: 69-74.
29. Thompson NE, Ketterhagen MJ, Bergdoll MS and Schantz EJ. (1984). Isolation and some properties of an enterotoxin produced by *Bacillus cereus*. *Infect. Immun.* 43: 887–894.
30. Wakayama S, Ishikawa F and Oishi K. (1984). Mycocerein, a novel antifungal peptide antibiotic produced by *Bacillus cereus*. *Antimicrob. Agents. Chemother.* 26: 939–940.
31. Naclerio G, Ricca E, Sacco M and Felice MD. (1993). Antimicrobial Activity of a Newly Identified Bacteriocin of *Bacillus cereus*. *Appl. Environ. Microbiol.* 59: 4313-4316.
32. Oscariz JC, Lasa I and Pisabarro AG. (1999). Detection and characterization of cerein 7, a new bacteriocin produced by *Bacillus cereus* with a broad spectrum of activity. *FEMS Microbiol. Lett*. 178: 337-341.

33. Ladeuze S, Lentz N, Delbrassinne L, Hu X and Mahillon J. (2011). Antifungal Activity Displayed by Cereulide, the Emetic Toxin Produced by *Bacillus cereus*. *Appl. Environ. Microbiol.* 77: 2555–2558.
34. Sadfi N, Cherif M, Hajlaoui MR, Boudabbous A and Belanger R. (2002). Isolation and partial purification of antifungal metabolites produced by *Bacillus cereus*. *Ann. Microbiol*. 52: 323-337.
35. Akhavansepahi A, Selselehzakeri S and Rezapanah M.R. (2011). Evaluation of antifungal activity of *Bacillus cereus* against *verticillium dahliae* and *phytophthora infestans* pathogenic plant fungi. *Journal of Sciences*; 20: 41-52.
36. Tschen JS and Tseng CN. (1989). Bacereutin, an antifungal antibiotic isolated from metabolites of *Bacillus cereus* CHU 130. Proc. *Natl. Sci. Counc. Repub. China B.* 13: 258-61.
37. Sunaina V, Ajay S and Dureja P. (2005). Bacterial metabolites from *Bacillus cereus* B4 responsible for potato plant growth. *Potato Journal*. 32: 187-188.