International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 10, 2016

Original Article

IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF BACTERIA HAVING ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY

GARIMA SHARMA, SHWETA DANG, SANJAY GUPTA, REEMA GABRANI*

Department of Biotechnology, Jaypee Institute of Information Technology, A-10, Sector 62, NOIDA, India
Email: reema.gabrani@jiit.ac.in

Received: 22 Apr 2016 Revised and Accepted: 12 Aug 2016

ABSTRACT

Objective: The aim of the current study was to isolate and identify the bacteriocinogenic strain exhibiting broad range antimicrobial activity and antibiofilm activity from the soil of animal farms.

Methods: In the current study, bacterial strains were isolated from soil of twelve different regions of the animal farm all over India and screened for antimicrobial activity against *Staphylococcus epidermidis*, *Micrococcus luteus, Pseudomonas fluorescence* and *Escherichia coli.* Antibiofilm ability of these selected strains was checked on preformed biofilm of *S. epidermidis* and in addition biofilm disruption potential was also determined. The potent bacterial strain was identified at the molecular level by 16S ribosomal DNA (rDNA) sequencing.

Results: 30 out of 231 strains isolated from soil were selected on the basis of antibacterial activity against *S. epidermidis.* One potential candidate (GAS 101) exhibited ≥99% inhibition against *S. epidermidis*, *M. luteus, P. fluorescence* and *E. coli* and also showed antibiofilm activity. GAS 101 16S rDNA sequencing data identified it as *Bacillus subtilis.* The sequence of *B. subtilis* was submitted to genbank under accession no. KJ564301.

Conclusion: *B. subtilis* GAS 101 isolated from soil of animal farm showed the antibacterial activity against all indicator organisms and also displayed antibiofilm activity against preformed biofilm and inhibited biofilm formation of *S. epidermidis*.

Keywords: *B. subtilis*, *E. coli, M. luteus*, *P. fluorescence*, *S. epidermidis*

DOI: http://dx.doi.org/10.22159/ijpps.2016v8i10.12338

INTRODUCTION

Bacterial nosocomial infections can pose a major risk mainly in immune-compromised patients. Advancement in medical sciences has led to the increased use of invasive devices resulting in high rates of infection [1]. Bacteriocins are ribosomally synthesized peptides which bacteria use as their natural defense mechanism in the same niche area. Bacteriocins have many properties like low toxicity, potency, narrow and broad spectrum effectiveness and *in situ* production. These properties indicate that bacteriocin can be used as alternate to antibiotic [2]. Production of bacteriocin is widespread in nature; a single bacterial species has been reported to produce 10 to 100 different types of bacteriocins [3]. Lactic acid bacteria (LAB) and *Bacillus* species both have gained the focus because of the ability to produce broad-spectrum inhibitory activity and GRAS status by the US FDA. Nisin and pediocin are the most studied antimicrobial substance from LAB which are commonly used in food production industry as a preservative agent and in the pharmaceutical industry as a health care product [4, 5].

An antimicrobial substance produced by *Bacillus* species may be placed on the second place after LAB because of the diversity in the antimicrobial peptide (AMP) due to different chemical structures [6]. The *Bacillus* subgroup has been reported to produce a large number of AMPs and this can be a good source to explore the novel antimicrobial substances [7, 8]. Subtilin synthesized by *B. subtilis* is a cationic AMP which belongs to lantibiotic and has been studied extensively. It has been shown to be active against broad spectrum gram positive bacteria and acts via pore formation [9]. A number of reported bacteriocins is very less as compared to the reported bacterial species, so necessitating a need to explore the properties and therapeutic applications of bacteriocins. The objective of the current study was to isolate the bacteria producing antimicrobial substance from soil of animal farm. The soil is a rich source of microorganisms, as it provides an enhanced environment for the growth of microbes, which contributes towards enormous diversity of bacteria. In the current study, we have isolated different microbes which are able to produce antimicrobial substances from the soil of animal farm using de Man, Rogosa and Sharpe (MRS) agar plates. The isolate that possessed the broad activity against indicator organisms and was able to disrupt biofilm was further identified by molecular characterization.

MATERIALS AND METHODS

Chemicals

The Chemicals and media used in the current study were procured from Hi-Media, CDH and Sigma-Aldrich.

Methods

Bacterial strains and growth conditions

Four different microorganisms *S. epidermidis* (MTCC no. 435), *P. fluorescence* (MTCC No. 2421), *M. luteus* (MTCC No. 106) and *E. coli* (MTCC No. 443) used in the current study were procured from MTCC, Chandigarh. All bacteria were cultured in nutrient broth at 37 °C for 16-18 h.

Isolation of antimicrobial substance producing bacteria from soil of animal farm

Bacterial strains were isolated from soil collected near the dairy of the various region of India (table 1). One gram of soil was mixed with 9 ml of autoclaved distilled water, homogenized using a vortex mixture and serially diluted with autoclaved water. Subsequently, 100 µl of each dilution was spread onto MRS agar plate (Hi-media) and the plates were incubated at 30 °C for 24 h. Well-isolated colonies were inoculated into fresh MRS broth for stock preparation.

Inhibition spectrum of cell free supernatant (CFS) of isolated bacterial strains

The inhibition spectrum of each isolate was examined using the 96 well microtiter plate assay against indicator organisms. The supernatant was obtained by centrifugation (10,000 ×g, 15 min at 4 °C) of culture grown in MRS broth at 30 °C for 24 h. CFS from overnight grown culture was incubated with the $1x10^6$ cfu/ml of the indicator organisms in each well of the 96 well plates [10]. Three wells in each

plate served as sterility control (without inoculum). Plates were incubated at 37 °C for 16-18 h and then examined for the growth inhibition with respect to control (indicator organism without CFS). The assay was carried out in triplicates and repeated thrice.

Determination of activity unit per ml (AU/ml)

Antimicrobial activity (AU/ml) was expressed as the reciprocal of the highest dilution that gave a definite zone of inhibition multiplied by a dilution factor [11]. $1x10^6$ cfu/ml of *S. epidermidis* was treated with the 1:2-1:128 dilution of the CFS from each isolate and incubated at 37 °C for 16-18 h. The highest dilution showing inhibition activity was considered as antimicrobial activity unit.

Antibiofilm activity of cell-free supernatant

CFS from each isolate was tested for its potential antibiofilm activity against preformed *S. epidermidis* biofilm and also on its biofilm formation.

Inhibition of cell attachment

CFS from each isolate was incubated with $1x10^6$ cfu/ml of *S. epidermidis* in the 96 well plate and incubated at 37 °C for 48 h to allow cell attachment and biofilm development. The plate was washed with phosphate buffer saline (PBS) and the crystal violet staining assay was performed [12]. Evaluation of % reduction in biofilm was evaluated by-

$$\% \text{ inhibition} = [1 - (\frac{\text{absorbance of treated bacteria}}{\text{absorbance of untreated bacteria}})] \text{ X } 100$$

Reduction of biofilm growth and development

The plates containing $1x10^6$ cfu/ml of *S. epidermidis* were incubated at 37 °C for 48 h to allow cell attachment and biofilm development. The wells were properly washed after incubation with PBS to remove unbound cells. CFS from each isolate was added to the well and further incubated for 24h. The plate was washed with PBS and crystal violet assay was performed as discussed above.

Molecular characterization and phylogenetic analysis of 16 S rDNA sequence of potential strain

Genomic DNA of the selected strain was isolated by cetyltrimethylammonium bromide (CTAB) method according to Cullings *et al.* 1992 [13]. The 16S rDNA of the strain (GAS 101) was amplified using universal primers 8-27F AGAGTTTGATCCTGGCTCAG and 1492R GGTTACCTTGTTACGACTTC; the sequence was submitted to Genbank (accession no. KJ564301). The phylogenetic tree of the amplified sequence of 16S rDNA of *B. subtilis* strains GAS101 with closely related *Bacillus* species was constructed by CLUSTAL W software.

RESULTS

Isolation of bacterial strains possessing antimicrobial activity

The isolation of bacterial strains from soil of animal farm possessing antimicrobial activity against gram positive and gram negative indicator bacteria was undertaken. Soil provides a natural habitat to bacteria and research shows that it is the well-reported resource for diverse microbiota [14]. Soil samples were collected in sterile conditions from twelve different regions of India (table 1). The colonies (231) obtained on the MRS agar plate were streaked again and transferred into MRS broth for isolating pure culture. These bacteria were initially screened for inhibition activity against *S. epidermidis* by checkerboard dilution method and 30 strains were selected for further study.

Table 1: Soil samples collected from different regions of India

Sample no.	Collection site	Total isolates	Isolates with antimicrobial activity
1	Ghaziabad (U. P.)	26	1
2	Ghazipur (Delhi)	33	10
3	Gurgaon (Haryana)	12	2
4	Bijnor (U. P.)	19	4
5	Agra (U. P.)	17	3
6	Mathura (U. P.)	35	9
7	Bulandsahar (U. P.)	17	1
8	Faridabad (Haryana)	22	0
9	Dehradun (Uttaranchal)	24	0
10	Sirdi (Maharastra)	14	0
11	Bhopal (M. P.)	17	0
12	Sikar (Rajasthan)	12	0
Total		231	30

Antimicrobial spectrum of cell free supernatant of selected strains

Antimicrobial activity of screened bacterial CFS was examined further against *P. fluorescence, M. luteus* and *E. coli* indicator organisms by 96 well microtiter plate assay to select the isolate possessing the broad range antimicrobial activity. The results of checker board assay indicates that 28 out of 30 strains showed >90% inhibition against *E. coli*, whereas 20 out of 30 showed inhibition of *P. fluorescence* cells and 19 strains were active against *M. luteus* (table 2). In totality 10 strains showed >90% inhibition against all chosen bacteria.

Determination of AU/ml

The AU/ml of CFS from all selected bacterial strains was checked against *S. epidermidis* as indicator organism (table 2). The activity ranged from 80 to 800 AU/ml and the highest activity was observed for GAS 101 strain up to 800AU/ml against *S. epidermidis.*

Antibiofilm activity of cell free supernatant

The antibiofilm activity of all the selected strains (30) after initial screening was checked against *S. epidermidis* biofilm. The capability of *S. epidermidis* to produce biofilm was checked on the congo red agar (data not shown). *S. epidermidis* is an opportunistic dermal pathogen which can cause infections on indwelling medical devices and surgical wounds by forming biofilm [15]. The capability of *S. epidermidis* to form biofilm complicates its removal as it is more resilient to eradication [16]. In the current study, the curing i.e. biofilm disruption as well as the preventive prospective, especially inhibition of biofilm formation, was investigated. Ten out of 30 chosen stains were potentially able to inhibit >90% *S. epidermidis* biofilm formation upon CFS incubation (table 2) and 4 strains could demolish the preformed biofilm by *S. epidermidis*.

Molecular characterization of potential candidate

The isolate which displayed antimicrobial activity against all selected strains, antibiofilm activity (formation and disruption) against *S. epidermidis* and demonstrated highest AU/ml was selected for molecular characterisation. The 16S ribosomal DNA was amplified by 16S universal primers and its DNA was sequenced for strain identification. The strain (GAS 101) was identified as *B. subtilis* strain and 16S rDNA partial sequence was submitted to GenBank (accession no. KJ564301). The phylogenetic tree of amplified sequence of 16 S rDNA was constructed and it showed high similarity with earlier submitted sequences of *Bacillus* subspecies (fig. 1).

Table 2: Brief summary of antimicrobial activity of cell free supernatant of isolates against various indicator organisms by the microtiter plate based antimicrobial assay and antibiofilm activity against *S. epidermidis* by crystal violet assay. Each value represents mean of three independent experiments carried out in triplicates±S. E., where n=3

S. No.	Isolate name	% inhibition against *S. epidermidis* MTCC 435	% inhibition against *E. coli* MTCC 443	% inhibition against *P. fluorescence* MTCC 2421	% inhibition against *M. luteus* MTCC 106	% inhibition of *S. epidermidis* biofilm formation	% inhibition of preformed *S. epidermidis* biofilm	AU/ml against *S. epidermidis*
1	GAS101	99.06±0.3	99±0.6	99.01±0.2	99.05±0.1	99.3±0.1	96.99±0.3	800
2	GAS 201	98±0.6	94±0.3	13.7±0.1	98.1±0.3	95.13±0.4	97.04±0.5	80
3	GAS 203	97.55±0.6	92.45±0.7	15.39±0.3	97.55±0.5	89.58±0.4	20.13±0.7	80
4	GAS 204	93.39±0.5	91.21±0.5	11.01±0.1	98.39±0.6	17.59±0.5	20.12±0.6	160
5	GAS 205	90.99±0.1	86.91±0.7	10.03±0.5	94.99±0.3	16.02±0.7	4.03±0.4	80
6	GAS 206	94.63±0.2	91.28±0.2	13.56±0.4	96.63±0.2	1.04±0.3	4.86±0.6	80
7	GAS 207	96.43±0.5	91.73±0.3	21.1±0.6	97.43±0.5	8.7±0.5	21.04±0.3	80
8	GAS 209	97.61±0.1	90.68±0.3	11.23±0.4	98.61±0.2	12.57±0.1	28.47±0.2	160
9	GAS 210	96.87±0.8	91.67±0.1	10.02±0.3	97.87±0.9	97.36±0.3	21.52±0.4	320
10	GAS 211	98.65±0.4	92.65±0.8	3.65±0.7	98.65±0.7	93.23±0.5	12.5±0.7	80
11	GAS 212	94.67±0.1	95.87±0.8	94.67±0.7	96.67±0.6	1.04±0.6	7.02±0.5	160
12	GAS 301	92.88±0.3	84.88±0.6	84.77±0.8	94.88±0.3	82.12±0.4	70.07±0.5	640
13	GAS 302	95.59±0.3	94.59±0.3	97.39±0.7	96.59±0.8	94.67±0.6	26.19±0.7	640
14	GAS 402	91.55±0.8	91.55±0.5	94.65±0.4	94.55±0.1	10.87±0.4	9.05±0.5	80
15	GAS 403	93.33±0.6	92.33±0.2	93.33±0.3	95.33±0.5	85.87±0.3	17.86±0.4	80
16	GAS 409	94.57±0.3	95.33±0.3	94.41±0.4	96.57±0.6	10.85±0.5	9.04±0.6	80
17	GAS 413	95.95±0.2	93.47±0.6	96.48±0.5	97.95±0.3	14.52±0.3	9.01±0.1	80
18	GAS 503	96.79±0.6	95.39±0.5	97.41±0.1	11.37±0.1	17.42±0.4	13.7±0.5	80
19	GAS504	96.89±0.4	94.65±0.1	97.39±0.6	16.44±0.6	3.21±0.3	13.5±0.1	160
20	GAS505	93.58±0.3	94.78±0.3	96.53±0.7	96.58±0.8	93.27±0.2	23.05±0.7	160
21	GAS601	94.59±0.1	95.43±0.1	95.51±0.4	96.59±0.5	93.21±0.3	23.01±0.5	160
22	GAS 602	94.89±0.1	94.89±0.3	95.81±0.3	12.36±0.4	2.01±0.1	6.43±0.4	80
23	GAS 603	91.55±0.3	92.55±0.7	94.75±0.1	14.37±0.1	92.72±0.4	15.91±0.6	80
24	GAS604	90.67±0.5	91.89±0.1	95.57±0.2	11.33±0.7	1.17±0.3	7.79±0.7	160
25	GAS605	90.79±0.4	91.88±0.2	95.19±0.5	15.21±0.2	99.02±0.5	12.74±0.9	160
26	GAS606	97.65±0.2	95.15±0.8	96.45±0.6	12.24±0.5	96.04±0.2	19.06±0.2	80
27	GAS607	94.65±0.1	95.65±0.7	95.05±0.5	11.27±0.7	4.03±0.1	90.05±0.6	160
28	GAS608	92.63±0.3	93.63±0.6	96.33±0.7	14.52±0.3	20.97±0.4	8.03±0.5	80
29	GAS609	95.46±0.5	95.46±0.6	96.66±0.8	12.35±0.2	20.77±0.3	15.73±0.1	160
30	GAS 701	97.54±0.6	95.54±0.2	95.5±0.1	13.36±0.7	2.64±0.5	99.02±0.4	80

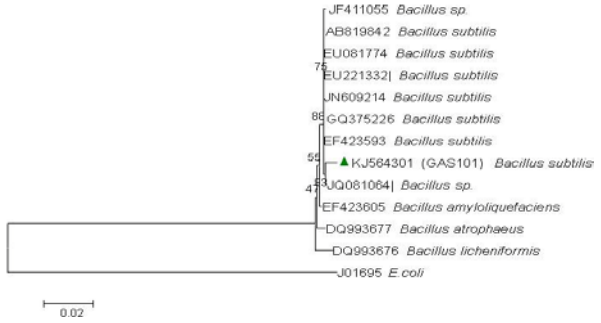

Fig. 1: Phylogenetic tree of partial sequence of 16S rDNA amplified from isolated *B. subtilis* GAS101 (KJ564301) constructed by Clustal W showing similarity with closely related *Bacillus* subspecies

DISCUSSION

Soil typically contains 10^9 to 10^{10} microorganisms per gram (dry weight) which may represent more than a million bacterial species [17]. Soil from animal farm remains rich in straw, dung, urine and traces of raw milk, all of these provide an absolute atmosphere to cultivate. Serially diluted soil sample was allowed to grow on the MRS agar plate. MRS agar is a selective medium containing sodium acetate which suppresses the growth of other competing bacterial group and favours the growth of lactic acid bacteria group and *Bacillus* species [18]. In preliminary selection, *S. epidermidis* was used as an indicator organism which is an opportunistic pathogen reported for causing infection. In the current study 30 isolates out of 231 showed >90 % inhibition against *S. epidermidis* and were further screened for broad range antibacterial activity. Other indicator organisms used in the current study belong to gram positive (*M. luteus*) and gram negative (*E. coli* and *P. fluorescence*) clan and have been reported to cause nosocomial infection [16, 19-21]. These infections persist and are resilient to antibiotic treatment. Ten strains showed>90% inhibition against indicator organisms in the current study which indicates towards the broader range of growth restriction. Nisin, Type A (I) lantibiotic, is FDA approved and has GRAS status which can restrict the growth of both gram positive and gram negative disease causing bacteria. Though nisin is approved as food biopreservative, it has proved its potential for other biomedical applications as well [22]. Apart from LAB, antimicrobial substance from *B. subtilis* has also been reported against the broader range of bacteria inclusive of *Salmonella*, *Bacillus cereus* and *Staphylococcus aureus* [23]. *B. subtilis* can produce wide array of antimicrobial substances having varied biochemical and biological properties.

S. epidermidis has the ability to colonize on the hospital device and form biofilm. Biofilm is multicellular, surface-attached film which provides architecture to microorganisms that contribute to resistance to antibiotics [16]. In the current study, two strains namely GAS 101 and GAS 301 demonstrated broad range antibacterial activity and in addition were able to inhibit *S. epidermidis* biofilm formation and showed disruption of biofilm. The molecular identification of strain GAS 101, with broader range of antimicrobial activity and highest (800) AU/ml against *S. epidermidis* showed this strain to be *B. subtilis*. CFS from isolated *B. subtilis* in the current study showed 800AU/ml against *S. epidermidis* whereas Cerein 8A, isolated from *Bacillus* species, was reported to have 400 AU/ml against *Listeria monocytogenes* [24]. BLIS extracted from *B. subtilis* BS 15 against *B. cereus* showed 100 AU/ml [25].

The chance of developing resistance to bacteriocins due to their diverse mechanism of action is less as compared to antibiotics [26]. Despite the huge potential, there are few reports available where

antimicrobial substances obtained from *B. subtilis* have been targeted against biofilm. Members of *Bacillus* group are considered good producers of diverse antimicrobial substances, including peptide, lipopeptide antibiotics and bacteriocins [6]. Bacteriocin from *Bacillus* species are of interest because of their broader range of activity including Gram negative bacteria and fungi [27]. Bacteriocin like inhibitory substance from *Bacillus* species has been reported for its antimicrobial activity and antibiofilm potential [28]. Sonorensin, a bacteriocin isolated from marine isolate *Bacillus sonorensis,* has exhibited *S. aureus* biofilm inhibition and since it could target both multiplying and non-multiplying bacteria, the resistance could not be developed against it [29]. Lipopeptides isolated from *B. subtilis* have shown the reduction in biofilm formation by 88% and dispersion of mature biofilm by 81% [30].

CONCLUSION

Aim of the current study was to isolate the strain with antimicrobial and antibiofilm potential against causative agents of nosocomial infection. Soil samples were collected from animal farm of twelve different regions. Total 231 isolates were preliminary screened against *S. epidermidis* and 30 isolates exhibiting more than 90% antimicrobial activity were selected. Selected strains were checked for their broader range of activity and the antibiofilm potential against *S. epidermidis* biofilm. Only one potential strain out of 30 showed>99 % inhibition against all indicator organism with highest activity unit (800AU/ml) against *S. epidermidis*. Molecular characterization by 16S rDNA sequencing of this strain showed it to be *B. subtilis*. In summary, antimicrobial substance isolated from the *Bacillus* species is a potential candidate as an antimicrobial and antibiofilm agent.

ACKNOWLEDGEMENT

We thank the Department of Biotechnology, Jaypee Institute of Information Technology, Noida, UP, India, for providing the infrastructural facility to carry out the research work.

CONFLICT OF INTERESTS

There is no conflict of interest

REFERENCES

1. Inweregbu K, Dave J, Pittard A. Nosocomial infections. Contin Educ Anaesth Crit Care Pain 2005;5:14-7.
2. Cotter PD, Ross RP, Hill C. Bacteriocins-a viable alternative to antibiotics? Nat Rev Microbiol 2013;11:95-105.
3. James R, Penfold CN, Moore GR, Kleanthous C. Killing of E. *coli* cells by E group nuclease colicins. Biochimie 2002;84:381–9.
4. Rajaram G, Manivasagan P, Thilagavathi B, Saravanakumar A. Purification and characterization of a bacteriocin produced by *Lactobacillus lactis* isolated from marine environment. Adv J Food Sci Technol 2010;2:138-44.
5. Vamanu E, Vamanu A. Viability of the *Lactobacillus rhamnosus* IL1 strain in simulated gastrointestinal conditions. Int J Pharmacol 2010;l6:732-7.
6. Stein T. *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. Mol Microbiol 2005;56:845–57.
7. Bizani D, Motta AS, Morrissy JA, Terra R, Souto AA, Brandelli A. Antibacterial activity of cerein 8A, a bacteriocin-like peptide produced by *Bacillus cereus*. Int Microbiol 2010;8:125-31.
8. Xie J, Zhang R, Shang C, Guo Y. Isolation and characterization of a bacteriocin produced by an isolated *Bacillus subtilis* LFB112 that exhibits antimicrobial activity against domestic animal pathogens. Afr J Biotechnol 2009;8:5611–9.
9. Teixeira ML, Dalla RA, Brandelli A. Characterization of an antimicrobial peptide produced by *Bacillus* subtilis subsp. spizezinii showing inhibitory activity towards *Haemophilus parasuis*. Microbiology 2013;159:980-8.
10. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. M7-A8. CLSI, Wayne, PA; 2009.
11. Ivanova I, Kabadjova P, Pantev A, Danova S, Dousset X. Detection, purification and partial characterization of a novel bacteriocin Substance produced by *Lactoccous lactis subsp. lactis* b14 isolated from Boza-Bulgarian traditional cereal beverage. Biocatalysis 2004;6:47-53.
12. Sharma G, Raturi K, Dang S, Gupta S, Gabrani R. Combinatorial antimicrobial effect of curcumin with selected phytochemicals on *Staphylococcus epidermidis.* J Asian Nat Prod Res 2014;16:535-41.
13. Cullings KW. Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. Mol Ecol 1992;1:233-40.
14. Maleki H, Dehnad A, Hanifian S, Khani S. Isolation and molecular identification of *Streptomyces spp.* with antibacterial activity from northwest of Iran. Biol Impacts 2013;3:129-34.
15. Ziebuhr W, Hennig S, Eckart M, Kränzler H, Batzilla C, Kozitskaya S. Nosocomial infections by *Staphylococcus epidermidis*: how a commensal bacterium turns into a pathogen. Int J Antimicrob Agents 2006;28:14-20.
16. Otto M. *Staphylococcus epidermidis*-the accidental pathogen. Nat Rev Microbiol 2009;7:555-67.
17. Eichorst SA, Breznak JA, Schmidt TM. Isolation and characterization of soil bacteria that define Terriglobus gen. nov., in the phylum *Acidobacteria*. Appl Environ Microbiol 2007;73:2708-17.
18. Mahrous H, Mohamed A, El-Mongy MA, El-Batal AI, Hamza HA. Study bacteriocin production and optimization using new isolates of *Lactobacillus spp.* isolated from some dairy products under different culture conditions. J Nutr Food Sci 2013;4:342.
19. Tagoe DNA, Baidoo SE, Dadzie I, Tengey D. Potential sources of transmission of hospital acquired infections in the volta regional hospital in Ghana. Ghana Med J 2011;45:22–6.
20. Obritsch MD, Fish DN, MacLaren R, Jung R. Nosocomial infections due to multidrug-resistant *Pseudomonas aeruginosa*: epidemiology and treatment options. J Pharmacother 2005;25:1353-64.
21. Bonjar S. Evaluation of antibacterial properties of some medicinal plants used in Iran. J Ethnopharmacol 2004;94:301-5.
22. Shin JM, Gwak JW, Kamarajan P, Fenno JC, Rickard AH, Kapila YL. Biomedical applications of nisin. J Appl Microbiol 2016;1201:1449-65.
23. Wu S, Jia S, Sun D, Chen M, Chen X, Zhong J, *et al.* Purification and characterization of two novel antimicrobial peptides subpeptin JM4-A and subpeptin JM4-B produced by *Bacillus subtilis* JM4. Curr Microbiol 2005;51:292–6.
24. Bizani D, Motta AS, Morrissy JA, Terra RM, Souto AA, Brandelli A. Antibacterial activity of cerein 8A, a bacteriocin-like peptide produced by *Bacillus cereus*. Int Microbiol 2005;8:125-31.
25. Alam SI, Kamran M, Sohail M, Ahmad A, Khan AK. Partial characterization of bacteriocin like inhibitory substance from *bacillus subtilis* BS15, a local soil isolate. Pak J Bot 2011;43:2195-9.
26. Guilhelmelli F, Vilela N, Albuquerque P, Derengowski LD, Silva-Pereira I, Kyaw C. In: Nadia SP, Octavio LF. editors. New edge of antibiotic development: antimicrobial peptides and corresponding resistance. Switzerland: Frontiers Media SA; 2013. p. 63.
27. Abriouel H, Franz CM, Omar NB, Gálvez A. Diversity and applications of *Bacillus* bacteriocins. FEMS Microbiol Rev 2011;35:201-32.
28. Korenblum E, Sebastián GV, Paiva MM, Coutinho CM, Magalhães FC, Peyton BM, *et al.* Action of antimicrobial substances produced by different oil reservoir *Bacillus* strains against biofilm formation. Appl Microbiol Biotechnol 2008;79:97–103.
29. Chopra L, Singh G, Jena KK, Sahoo DK. Sonorensin: a new bacteriocin with potential of an anti-biofilm agent and a food biopreservative. Sci Rep 2015;21:5.
30. Moryl M, Spętana M, Dziubek K, Paraszkiewicz K, Różalska S, Płaza GA, *et al.* Antimicrobial, antiadhesive and antibiofilm potential of lipopeptides synthesised by *Bacillus subtilis*, on uropathogenic bacteria. Acta Biochim Pol 2014;62:725-32.

How to cite this article

- Garima Sharma, Shweta Dang, Sanjay Gupta, Reema Gabrani. Identification and molecular characterization of bacteria having antimicrobial and antibiofilm activity. Int J Pharm Pharm Sci 2016;8(10):111-114.