Broad spectrum anti-microbial compounds producing bacteria from coast of Qingdao bays

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Abstract: Anti-microbial resistance burden and hazard associated with chemical treatment of infections demanded for new anti-microbial natural products. Marine associated microorganisms are the enormous source of bioactive compounds. In this study we have isolated 272 marine bacteria among them 136 (50%) were antagonistic to at least one of the four pathogenic strains *Listeria monocytogenes, Vibrio cholerae, E. coli* and *S. aureus*. Only two strains exhibited antibacterial activity against all four test strains, which were identified by 16S rDNA sequencing as *Bacillus sp.* DK1-SA11 and *Vibrio sp.* DK6-SH8. Marine isolate DK1-SA11 has potential to resist boiling temperature and pH 2-12. Furthermore cell free extract (CFE) inhibited all test organisms including superbug MRSA and pathogenic yeast *Candida albicans.* Marine isolate *Bacillus sp.* DK1-SA11 could be a potential combatant for the battle of drugs and bugs.

Keywords: Broad spectrum; marine bacteria; anti-microbial; MRSA; *Bacillus sp*.; *Vibrio sp*.

INTRODUCTION

After the discovery of penicillin, a miracle drug, the pathogenic microorganisms start developing resistance against antibiotics and chemotherapeutics agents. Recently, emergence of multiple drug resistant (MDR), methicillin-resistant *Staphylococcus aureus* (MRSA) and other superbugs increases the uncertainty for the use of antibacterial drugs in future. Moreover, increase in MIC (minimum inhibitory concentration) of teicoplanin and vancomycin against several strains of MRSA has been stated in many international hospitals (Tan and Zou, 2001). In United Sates annually ~19,000 mortality are due to MRSA (Klevens *et al.*, 2007). Moreover, the bacterial resistance ratio in China reaches to almost 90% among nosocomial infections (Benquan *et al.*, 2002; Zhang *et al.*, 2006). Emergence of MDR, extensively-drug-resistant (XDR) and pan-drug-resistant (PDR) superbugs, including *Acinetobacter baumannii, Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are considerable threats to public health (Peleg and Hooper, 2010; Pages *et al.*, 2008). Hence, discovery or developments of new and alternative therapeutic agents become imperative to overcome the resistance hazards (Nascimento *et al.*, 2000).

More than 50% of bacterial isolates from marine sources show antimicrobial activity against other microorganisms. Interaction between or among bacteria is very diverse, and being studied in various microparticles. The alteration in structures and functional activities of microbial community occur when antimicrobial interactions take place (Long and Azam, 2001). Morbidity or mortality of microbes results from antibiotic effect produced by microorganism to compete for available nutrients and breathing space, which are survival of the fittest strategies by microorganism. Therefore, bacteria associated with oceanic environments are intact source of novel anti-microbial compounds, because, at first, marine microorganisms have developed distinctive survival means against stress of temperature, salinity, nutrient depletion and pressure in the oceans, this results in altered physical and biochemical behaviors; secondly, food and living space contest among microbes in some oceanic bays predominantly of the particle attached microbes and sediment living bacteria has developed a competition of bacterial selection towards new survival approaches and the production of new compounds; Thirdly few bacterial floras have obtained unique defense techniques by formation of new metabolites which may be useful for protection from predators like protozoa (Zheng *et al.*, 2005; de Carvalho and Fernandes, 2010; Utsui and Yokota, 1985; Schaberle *et al.*, 2010). About 10^{29} marine bacterial cells are present in oceanic world (Whitman *et al.*, 1998). Moreover, in past 50 years about 10,000 and counting metabolites from ocean have been isolated and its properties were studied, among these bioactive metabolites 18% were acquired from marine bacteria (Bhatnagar and Kim, 2010). It has also been reported that there are about 38% annual increases in novel natural products from marine microbial sources (Blunt *et al.*, 2013). However, there are very large areas of marine microbial world, which is still unknown and only < 0.1% ocean biosphere was covered for marine microbiology (Ramaiah, 2004).

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The metabolites produced by marine bacteria have unique chemical structure characteristics compared to compounds produce by soil microorganisms (Trujillo *et al.*, 2007; Nascimento *et al.*, 2000; Woodford, 2005). Therefore, antagonistic marine microorganisms has been studying since 1947, when Rosenfeld and Zobell (1947) first time in the history established that anti-microbial metabolites can also be produced by marine bacteria which were antagonistic to pathogenic bacteria. The researcher began to sieve and search ocean to isolate and identify novel bioactive compounds from the early 1960s and the first marine origin bacterial antibiotic was isolated in 1966 (Burkholder *et al.*, 1966; Bhatnagar and Kim, 2010). The green control of infections by natural bacterial isolates has clear advantage over chemical treatments, which give enormous environmental pollution (Yebra *et al.*, 2004). Numerous novel anti-microbial agents against superbugs including MRSA are reported from marine resources, which were isolated from marine bacteria including *Pseudoalteromonas*, *Pesudomonas*, *Bacillus*, *Marinospora* and *Streptomyces* (Rahman *et al.*, 2010). However, many antibiotics are becoming ineffective due to evolution of antibiotic resistance in pathogenic bacteria (Taubes, 2008). Therefore, the objective of this study was to isolate and pool out the broad-spectrum anti-microbial compounds producing bacteria from marine resources. This study will help to isolate potential bacterial candidate (s) to overcome the needs of anti-microbial resistance battle between drugs and bugs.

MATERIALS AND METHODS

Sampling

Sampling of sea invertebrates, mangroves and fishes were collected from 8 different locations of costal area of Qingdao bays in Yellow sea (fig. 1) and from fish market during October 2011 to June 2012. Before sampling ice box were disinfected with 70% ethanol. All samples were aseptically transferred into sterile plastic bags and immediately place at disinfected icebox. All samples were stored at 4°C till further use.

Isolation of bacterial flora from marine samples

Each sample was weigh aseptically and put into sterilized seawater (~1:10), and then mixed by vigorous shaking. Ten fold serial dilutions were made up to 10^{-6} in sterile seawater and 0.1mL of each dilution was spread on marine agar 2216. All plates were incubated at 30°C for 48-72 hours. After 48 hours onward colonies with different morphology were selected and purified by subculturing. All pure isolated colonies were preserved in marine broth 2216 and BHI broth with 30% glycerol in corning cryogenic tubes and store in -80°C.

Screening of bacterial isolates for Antibacterial activity

All isolated colonies were assayed by modified double agar layer method (Dopazo *et al.*, 1988). Each isolated colony was spotted by sterile wooden stick separately on marine agar 2216 plates, with the help of colony counting grid at the back of plate, in five duplicates. All plates were incubated at 30°C for 3 days to produce macro-colonies. About 18 hours old culture of test organisms *Listeria monocytogenes* (NCMCC 54001), *Vibrio cholerae* (ATCC 14035) *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538) were mix separately in (0.7%) BHI agar with 0.2 optical density OD_{600} (0.5 McFarland, 10^8 cfu/mL) maintained at 45°C. Next, test cultures were carefully pour on macro-colonies plates separately. One set of isolates plates were layered by sterilized semisolid BHI agar as negative control to check possible spread of isolated colonies on top layer. After solidify all plates were place at 4°C for 4 hours for diffusion of antibacterial compound in top agar layer, then test strains *L. monocytogenes* plates were incubated at 30°C and rest of three test strain plates were incubated at 37°C for 24 hours and zone of inhibition around macro-colonies of marine isolates were measured.

Preparation of cell free extract

Marine isolates DK1-SA11 and DK6-SH8 were culture separately in 3mL marine broth 2216 for 24 hours at 28°C with 120 rpm rotation then transfer in 100mL broth for 72 hours at 28°C with 120 rpm rotation. Cells were separated via centrifugation (Sigma) at 9000 g for 15 min at 4°C followed by filtration through 0.22µm filters (Millipore), and then cell free supernatants (CFS) were lyophilized at -90°C under negative pressure. Cell free extract (CFE) powders were weighed aseptically and store in sterile tubes at -20°C. Medium without bacterial inoculation, marine broth 2216 extract (MBE) was extracted as negative control.

Antibacterial activity of CFE

Antibacterial profile of both isolates was assayed by oxford cup diffusion inhibition assays (Zhou *et al.*, 2006) with modifications. CFE harvested from DK1-SA11 and DK6-SH8 was prepared 0.1 mg/mL in Mili-Q water and pH 7.0 was adjusted with 1M NaOH and 1M HCl to prevent the inhibitory effect of pH. All test strains were grown in BHI broth (except yeast was grown in YPD broth) overnight diluted into same broth to reach 0.2 OD_{600} approximately 10^8 cfu/mL and spread on 0.7% BHI agar (YPD agar for yeast) plates by sterile cotton stick; sterile Oxford cups were put on the top of agar with test strain, and 100 µl of 0.1 mg/mL CFE were pour into each Oxford cup. The plates were then placed at 4°C for 2 hours for diffusion; then plates were incubated for 24 hours at 37, 30 & 25°C depending on the indicator organisms. Negative control of 0.1mg/mL of MBE; 10µl of 10mg/mL neomycin (Sigma-Aldrich) and 10µl of 100µg/mL ampicillin (Sigma-Aldrich) were used as positive control. Clear zone around the Oxford cup in the test strain lawn were regarded as antagonist activity of CFE (Messi *et al.*, 2001). The inhibition zone diameter (mm) was measured by caliper in triplicates.

Fig. 1: Sampling Sites of coast of Qingdao city Bays; from each site two samples were collected in different period

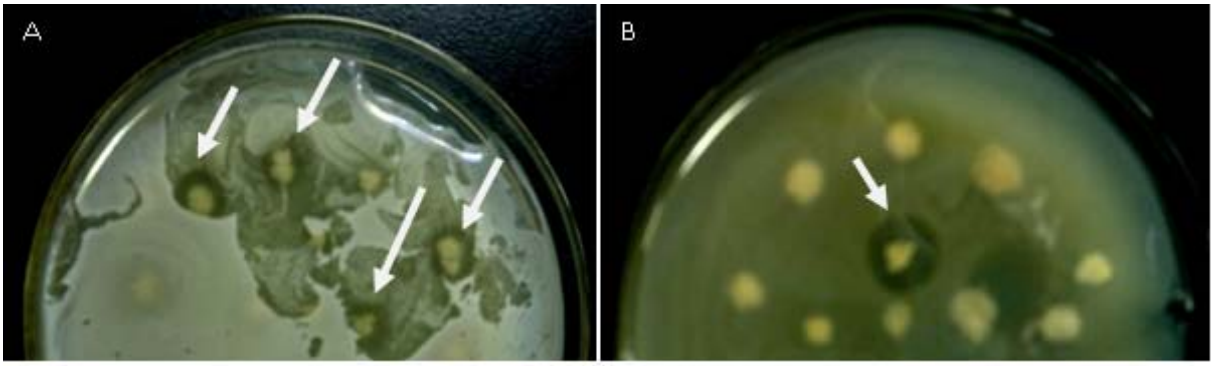

Fig. 2: Zone of inhibition after screening by double agar layer method (A) against *E. coli* (B) against *S. aureus* white arrows shows zone of inhibition around colonies

Thermal and proteolytic stability of CFEs

Thermal shock stability of both CFEs were assayed by treating 1mL of 0.1mg/mL CFE solution for 10 min at 40°C, 60°C, 80°C, 100°C and 121°C for 15 min (van Reenen *et al.*, 1998).

Proteolytic stability was analyzed as describe previously (Mirhosseini *et al.*, 2008). Untreated sample were used as controls. Proteolytic effect was determined by absence of an inhibition halo around enzyme treated oxford cup (Barja *et al.*, 1989). The antibacterial activities of treated CFEs were measured against *S. aureus* (ATCC 6538).

Effect of pH on CFEs

CFEs solutions of 0.1g/mL were prepared in each buffer (pH 2-12) and incubated at room temperature for 12 hours. CFEs solutions were then adjusted to pH 7 (van Reenen *et al.*, 1998), and activities were tested against *S. aureus* (ATCC 6538). CFEs in Mili-Q water 0.1 g/mL was used as control.

Identification of marine isolates strain and phylogeny analysis

Marine isolates DK1-SA11 and DK6-SH8 were identified to the genus level by studying of phenotypic

characteristics (Gram *et al.*, 2010) and 16S rRNA gene sequencing. Genomic DNA was extracted from the marine isolates by UNIQ-10 Spin Column Bacterial Genomic DNA Minipreps Kit (Sangon Biotech; Shanghai). The 16S rRNA genes were amplified via universal primers 27F (AGAGTTTGATCMTGGCTCA-G) and 1492R (TACGGYTACCTTGTTACGACTT) (Gram *et al.*, 2010), primary DNA denaturation at 95°C for 5 min, go after by 30 cycles of 94°C for 60 sec, 55°C for 60 sec, and 72°C for 90 sec, final extension for 10 min at 72 °C. PCR was performed in a Peltier Thermal cycler-200 (Bio-Rad) (Pinto *et al.*, 2009). Following amplification, 10µl of PCR products were separated at 100 V for 30 min in a 0.7 % (w/v) agarose gel in 1× TBE buffer. A 1-kb ladder molecular weight marker (TaKaRa Biotechnology; Dalian) was used. Agarose gel was visualized under Tanon® 4200F gel documentation system. The PCR products were purified and sequenced by BGI Qingdao (China). The sequences were compared in standard nucleotide BLAST analysis of GenBank, NCBI database (Nithyanand and Pandian, 2009). Phylogeny tree was assembled with software MEGA version 4.0 by neighbor joining method (Tamura *et al.*, 2007). Bootstrap analysis for 1000 replicates was performed to estimate reproducibility of the tree topologies. The analyzed sequences have been deposited in the GenBank databases.

STATISTICAL ANALYSIS

All experiments were design randomly in triplicates. The combination groups of test strains were calculated by ${}^{n}C_{r}$. Values in tables are expressed in mean ± SEM (standard error mean). The software GraphPad Prism® (v 5.01) was used for statistical analysis of data.

RESULTS

Isolation and Screening of bacterial isolates for antibacterial activity

About 272 bacterial isolates were isolated and preserve in medical freezer at -80°C. Isolates which have ≥ 1 mm zone of inhibition around macro-colonies were considered as antagonistic to test strains (fig. 2). About 50% of all isolates shows antagonistic activity at least one of four test strains (LM) *L. monocytogenes* (NCMCC 54001), (VC) *V. cholerae* (ATCC 14035), (EC) *E. coli* (ATCC 8739) and (SA) *S. aureus* (ATCC 6538) (table 1). Only two (0.7%) isolates, DK1-SA11 and DK6-SH8, were exhibited capability of producing antibacterial compounds to all four strains SA, EC, LM & VC (fig. 3). These two isolates have been selected for further studies. Phenotypic and genotypic 16S rDNA PCR amplification (fig. 4) and sequencing followed by phylogeny tree study were identified isolates DK1-SA11 as *Bacillus sp.* (fig. 5) and DK6-SH8 as *Vibrio sp.* (fig. 6).

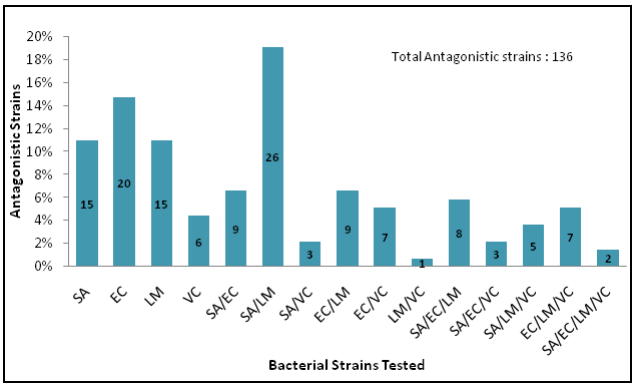

Fig. 3: Marine isolates showing antagonistic activity against tested organism SA (*Staphylococcus aureus*), EC (*E. coli*) LM (*Listeria monocytogenes*), VC (*Vibrio cholerae*) Value inside the percent bar represents the number of antagonistic isolates

Anti-microbial activity of CFE

Anti-microbial activities of cell free extract were tested against standard pathogenic strains obtained from different collection centers. CFEs of DK6-SH8 & DK1-SA11 were active against *E. coli*, *L. monocytogenes*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, *Shewanella putrefaciens*, *S. aureus* and *V. cholerae*. Furthermore, CFE of DK1-SA11 was also antagonistic to *Klebsiella pneumoniae*, MRSA, *Shigella sonnei*, both stains of *V. parahaemolyticus* as well as pathogenic yeast *Candida albicans*. Zone of inhibition by CFE of DK1-SA11 was greater than DK6-SH8 (table 2).

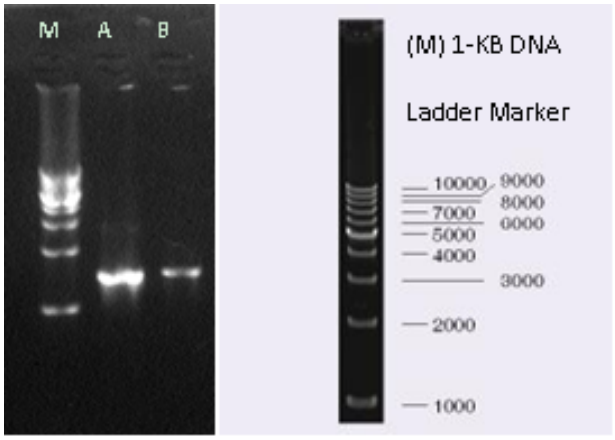

Fig. 4: PCR product bend of 16S rDNA of isolates (A) DK1-SA11 and (B) DK6-SH8 (M) 1-KB Marker

Thermal, proteolytic and pH stability of CFE

Both CFEs solutions of isolates DK1-SA11 and DK6-SH8 were active after 10 min heat shock at 40°C. CFE of DK6-SH8 lost its activity on temperature above 60°C, while CFE of DK1-SA11 was stable to thermal shock and there was no remarkable change in inhibition zone at temperature 60°C, 80°C & 100°C (fig. 7), although

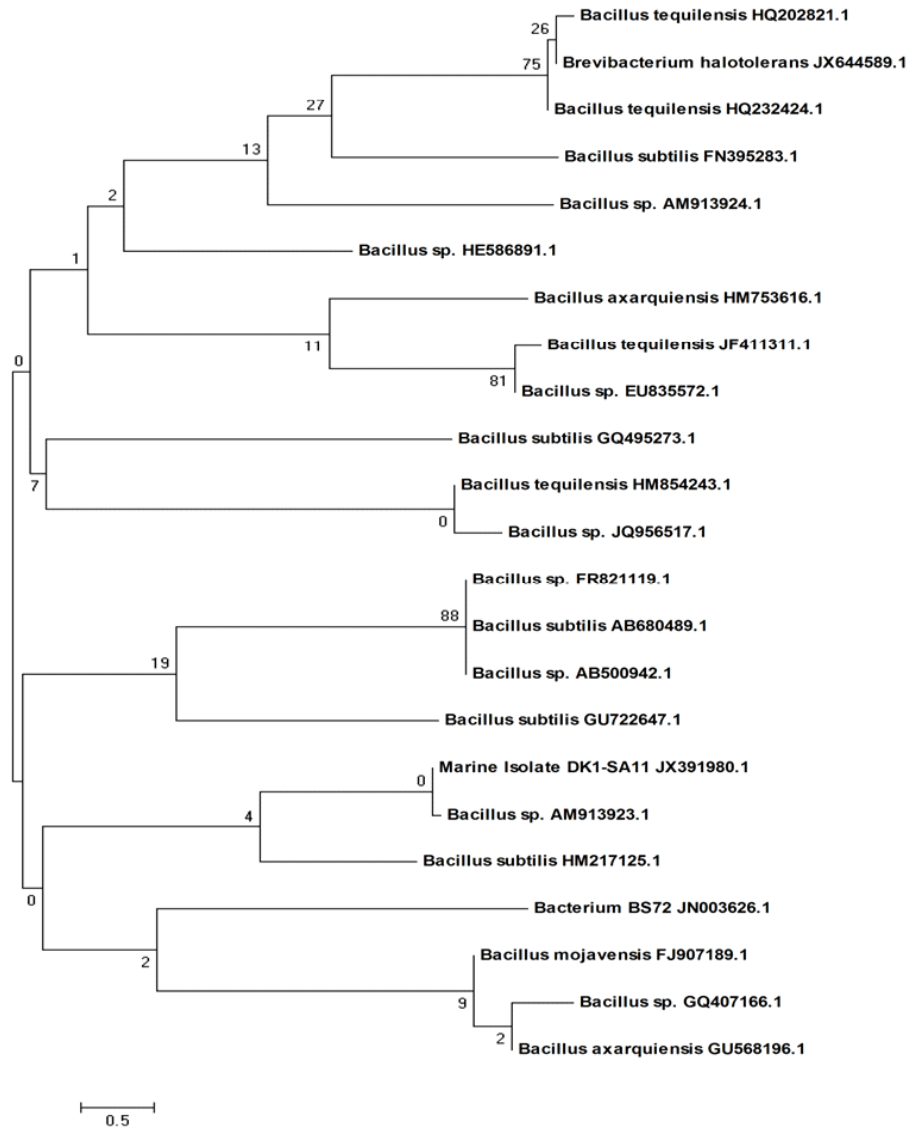

Fig. 5: Phylogenetic tree of marine isolate DK1-SA11

activity was lost after autoclaving at 121°C for 15 min. There was no activity seen after trypsin treatment by both isolates CFEs. CFE of DK6-SH8 lost its activity after treatment with pH buffer blow pH-5 and above pH-8, although DK1-SA11 CFEs shows stable activity after treating with pH 2-12 (fig. 8).

DISCUSSION

Yellow sea bays in Qingdao, China lies in temperate zone, and is very natural and famous among tourists. In this study our objective is to discover anti-microbial producing bacteria from marine resources. As the surface of marine organism are rich of nutrients than immoveable resources and sea water (Sponga *et al.*, 1999), it has been reported in many studies that surface attached colonized bacteria have more anti-microbial producer as compare with free live bacteria in sea (Armstrong *et al.*, 2001). One the basis of these reports, we have isolated 272 bacteria from 21 different samples from surface of marine fishes, invertebrates and seaweeds. Our findings are in agreement with previous reports; 50% (136 out of 272) of isolates having antagonistic effect to at least one of the four target strains tested. Irina Alekseevna *et al.* (2013) also reported 68.97% bacterial isolates from temperate zone of sea with anti-microbial activity. Bacteria growing on surface of marine organism are not really symbiotic but can be term as associated bacteria (Bultel-Ponce *et al.*, 1999) with consanguineous connection with host. These bacteria produce metabolites including antibiotic, toxins and amino-acids favorable for production and metabolism of host concern, as well as to enhance chemical defense capacity. On other hand, bacteria gains essential nutrients including fatty acids, polysaccharides and vitamin from

animal or plant hosts (Armstrong *et al.*, 2001). In our study the anti-microbial spectrums of activity of antagonistically active bacterial isolates have diverse combination against test strains. Among 136 anti-microbial active isolates 15 (11%) isolates shows antagonistic activity against *S. aureus* (SA), 20 (14.7%) were active against *E. coli* (EC), 15 (11 %) were active against *L. monocytogenes* (LM), 6 (4.4%) active against *V. cholerae* (VC); isolates antagonistic to two test strains were, 9 (6.6%) active against both SA & EC, 26 (19.1%) active against SA & LM, 3 (2.2%) against SA & VC, 9 (6.6%) against EC & LM, 7 (5.1%) active against EC & VC, 1 (0.7%) was antagonistic to LM & VC; 8 (5.9%) were active against three test strains including SA, EC & LM, 3 (2.2%) were active against SA, EC & VC, 5 (3.7%) were antagonistic to SA, LM & VC, 7 (5.1%) were active against EC, LM & VC and 2 (1.5%) marine isolates DK1-SA11 and DK6-SH8 were active against all four strains SA, EC, LM & VC (fig. 3). Our findings were similar to Zheng *et al.* (2005) who also reported varied anti-microbial spectrum of activity by marine isolated bacterial strains: Out of 42 antagonistic strains, 35 (83%) were antagonistic to *Agrobacterium tumefaciens*, 29 isolates (69%) exhibit antagonistic to *Bacillus subtilis*, 22 (52%) were antagonistic to SA, 8 (19%) were antagonistic to *Saccharomyces cerevisiae*, 4 isolates (9%) were antagonistic to EC and only four strains were active against all tested microorganisms.

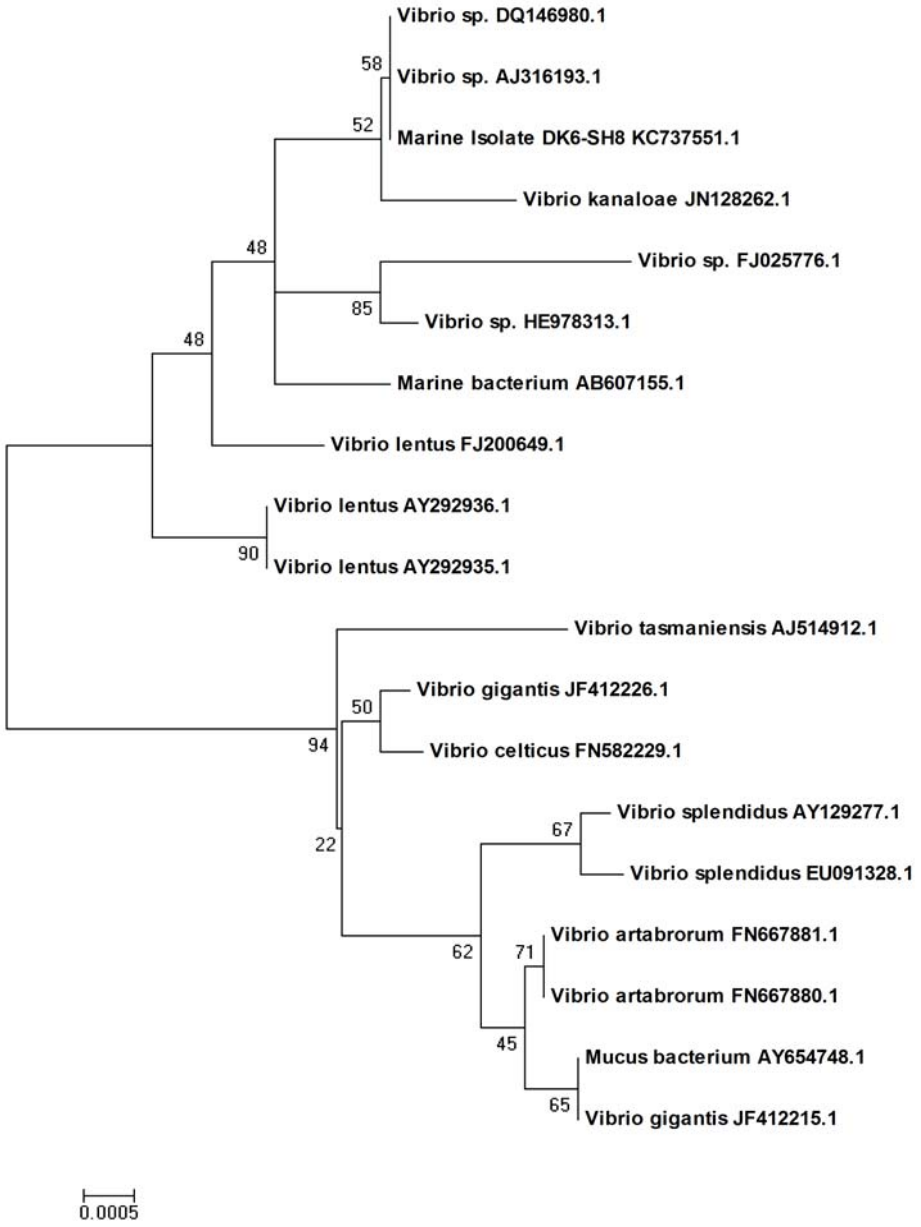

Fig. 6: Phylogenetic tree of marine isolate DK6-SH8

Table 1: Sampling collection particulars and number of antagonistic bacterial isolates from each sample

Sample ID	Sample types	Sampling months	Sampling locations			Total bacterial count (cfu/mL)	Isolated Strains	
			Label	Latitude	Longitude		*Antagonistic isolates	Total
DK1	Shell	Oct-2011	Qingdao bay	36.059854	120.309563	4.8×10^7	10	11
DK2	Squid	Oct-2011	Qingdao bay	36.05923	120.320978	4.8×10^6	8	10
DK3	Shell	Oct-2011	Huiquan bay	36.05694	120.333166	6.0×10^6	6	10
DK4	Shell	Oct-2011	Huiquan bay	36.054512	120.339003	5.0×10^6	4	9
DK5	Crab	Nov-2011	Taiping bay	36.048058	120.341921	9.6×10^7	7	10
DK6	Crab	Nov-2011	Taiping bay	36.049307	120.346642	6.8×10^7	9	10
DK7	Oyster	Nov-2011	Botanical garden Coast	36.048891	120.361748	9.0×10^6	8	10
DK8	Shell	Nov-2011	Botanical garden Coast	36.054512	120.364151	7.7×10^7	10	10
DK9	Sea weeds	Apr-2012	Qingdao bay	36.06041	120.312824	1.7×10^7	8	10
DK10	Oyster	Apr-2012	Qingdao bay	36.061173	120.31703	3.6×10^7	0	10
DK11	Fish	Apr-2012	Huiquan bay	36.056524	120.332136	3.1×10^6	6	10
DK12	Sea weeds	Apr-2012	Huiquan bay	36.052777	120.339518	9.3×10^7	3	10
DK13	Snail	May-2012	Taiping bay	36.049377	120.349731	5.5×10^7	2	10
DK14	shell	May-2012	Taiping bay	36.049862	120.353851	7.1×10^7	7	10
DK15	Sea weeds	May-2012	Botanical garden Coast	36.050209	120.361233	5.6×10^7	1	10
DK16	Fish	May-2012	Botanical garden Coast	36.053332	120.363035	9.5×10^6	0	10
FM1	Prawn	Oct-2011	Fish Market-1	-	-	8.8×10^6	12	20
FM2	Crab	Oct-2011	Fish Market-1	-	-	1.9×10^7	9	20
FM3	Shell	Oct-2011	Fish Market-1	-	-	2.4×10^7	9	24
FM4	Prawn	Nov-2011	Fish Market-2	-	-	5.9×10^7	3	28
FM5	Sea weeds	Nov-2011	Fish Market-2	-	-	3.9×10^6	14	20
						Total	136	272

*Antagonistic to at least one of the test the test strains.

Table 2: Antimicrobial activity of CFE from selected isolates

Microorganism	Strain	Zone of inhibition in mm ± SEM*			
		CFE of DK1-SA11	CFE of DK6-SH8	Ampicillin	Neomycin
Candida albicans	ATCC 10231	15 ± 0.34[a]	R	-	-
E. coli	ATCC 8739	15 ± 0.89[b]	13 ± 0.34[b]	24±0.89[a]	R
Klebsiella pneumoniae	NTCC9133	18 ± 0.58[a]	R	13 ± 0.89[b]	11±0.34[b]
Listeria monocytogenes	NCMCC 54001	16 ± 0.34[b]	12 ± 0.34[c]	22 ± 1.77[a]	17±0.89[b]
MRSA	ATCC 43300	18 ± 0.34[a]	R	R	R
Pseudomonas fluorescens	CCTCC AB2010226	16 ± 0.34[b]	12 ± 0.67[c]	21 ± 0.58[a]	13±0.34[c]
Salmonella typhimurium	ATCC14028	20 ± 1.16[a]	11 ± 0.34[b]	R	R
Shewanella putrefaciens	CCTCC AB2010225	16 ± 0.34[b]	12 ± 0.34[c]	27 ± 0.89[a]	15 ±0.58[b]
Shigella sonnei	ATCC 25931	19 ± 0.34[b]	R	24 ± 1.77[a]	12 ± 0.58[c]
Staphylococcus aureus	ATCC6538	18 ± 1.16[b]	12 ± 0.58[c]	33 ± 1.74[a]	12 ± 1.21[c]
Vibrio cholerae	ATCC 14035	15 ± 0.34[b]	12 ± 0.34[c]	38 ± 1.46[a]	16 ± 1.16[b]
Vibrio parahaemolyticus	ATCC17802	20 ± 0.34[a]	R	21 ± 0.34[a]	13 ± 1.00[b]
Vibrio parahaemolyticus	ATCC33847	18 ± 1.46[a]	R	20 ±1.21[a]	13 ± 0.89[b]

MRSA (Methicillin resistant *staphylococcus aureus*)
CFE (Cell free extract)
R (Resistant)
* Mean of triplicates ±Standard Error Mean
Alphabets (a,b.c) superscripts in rows shows significantly different ($p<0.05$)

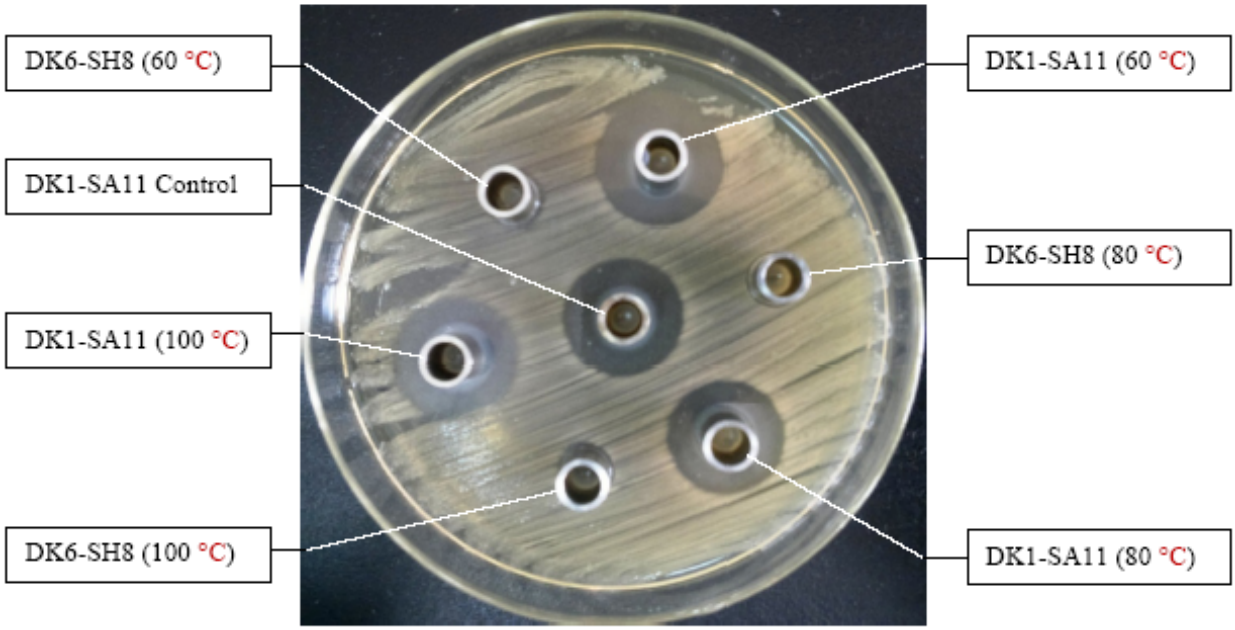

Fig. 7: Zone of inhibition by CFEs after Heat shock against *S. aureus*

CFEs of *Bacillus sp.* DK1-SA11 and *Vibrio sp.* DK6-SH8 exhibit broad spectrum of activity against test organisms (table 2). Irina Alekseevna *et al.* (2013) have isolated marine vibrios with broad spectrum of antibacterial metabolites, our isolate *Vibrio sp.* DK6-SH8 can inhibit 7 test strain out of 13 bacterial strain tested, which is in accordance with previous reports. The anti-microbial substance by both isolates seems to be protein in nature as inactivated by trypsin treatment. Furthermore, DK1-SA11 CFE is more stable at heat and pH treatments, which makes it highly potential candidate for anti-0microbial agents. The spectrum of activity of DK1-SA11 against all tested organism, including yeast pathogen *Candida albicans* (ATCC 10231) and MRSA (ATCC 43300), appears to be the possible solution for antibiotic resistance by pathogens. Broader spectrum of activity by *Bacillus sp.* strain FAS_1 was also reported by Moshafi *et al.*

(2011), their isolate can inhibit all test strains including bacteria and fungi.

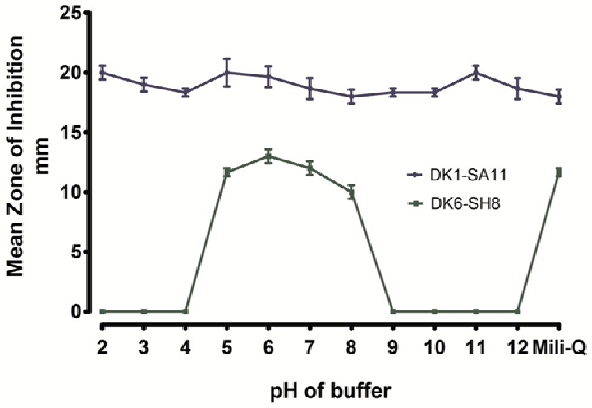

Fig. 8: Effect of pH buffer on antagonistic activity against *S. aureus.* Each point represents Mean ± SEM (including 8 mm oxford cup diameter)

CONCLUSION

We have isolated 136 potential anti-microbial compounds producing marine bacteria, among them *Bacillus sp.* DK1-SA11 (accession number: JX391980.1) and *Vibrio sp.* DK6-SH8 (accession number: KC737551.1) were identified and anti-microbial spectrum of their CFEs were checked against potential harmful bacteria. The broad spectrum antagonistically active marine isolate DK1-SA11 could be a potential troop for producing anti-microbial drugs, which can achieve edge against bugs. Further studies on *Bacillus sp.* DK1-SA11 are required to identify the anti-microbial compounds.

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