

## Isolation and Molecular Level Identification of DNase Producing Halophilic *Bacillus cereus* Family Isolates from Marine Sediment Sample

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### Abstract

Halophilic bacteria are excellent source of enzymes not only salt stable but also it can withstand and carry out reactions very efficiently under extreme conditions. Screening of bacteria from different hyper saline environment in Kanyakumari Coast, South India led to the isolation of total 111 culturable heterotrophic bacteria. Screening of Halophiles for the DNase production led to 23 isolates and from which maximum DNase producing 4 strains were selected through DNase well diffusion method. The potential isolate KVCMST-8A 12 showed maximum zone of inhibition at 35mm after 72 hours, which were able to grow optimal in media with 2-40% of salt. According to its phenotypic characteristics and comparatively partial 16S rRNA sequence, the halophilic KVCMST-8A 12 was identified as member of *Bacillus cereus* family.

**Keywords:** DNase, Halophilic, *Bacillus cereus*, *In vitro* assay, Substrate gel

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## INTRODUCTION

Marine environment is the prime reservoir of biological diversity and the marine microorganisms are recognized to be rich source of novel compounds. In India about 1000 natural products were derived from marine microbes (Suthindhiran *et al.*, 2010). Marine microorganisms are mostly unexplored and marine environment is rich source of microorganisms producing novel and efficient compounds (Sujatha *et al.*, 2005). Microbes form the topmost source of industrially important enzyme producers due to their rapid doubling time when compared to plants or animals (Kumar and Takagi, 1999). The ability of marine bacteria to synthesis variety of enzymes with good stability at higher temperature, alkaline conditions and high salinity (Berdy 2005) are attractive phenomenon to accomplish extremophilic organisms in biotechnological processes.

DNase catalyses the hydrolysis of deoxyribonucleic acid by breaking down phosphodiester bonds (Nishino and Marikawa, 2002), consequently DNase is considered to have a potential role in DNA utilization and nutrient cycling in the environment (Mulcahy *et al.*, 2010). The balance of extracellular DNA in the marine environment is largely regulated by DNase, which is important for the functioning of deep sea ecosystems (Dell'anno and Danovaro 2005). The role of DNases in cellular metabolism of nitrogen and phosphorous suggests that DNase secretion may also play an important role in the fate of extracellular DNA in the natural environment, particularly with regard to the dynamics and stability of marine aggregates (Aisha *et al.*, 2019).

Halophiles are the most likely source of enzymes, because not only is their enzyme salt tolerant but also may be active at high temperature, and pH values (Gomez and Steiner 2004). The isolation of moderate halophiles with capacity to produce extracellular enzymes will provide the possibility to have optimal activities at different salt concentrations (Ventosa *et al.*, 1998). Halophiles are now gaining more access to industrial microbiology and biotechnology because halophiles grow at high salt concentration and this minimize the risk of contamination during cultivation (Oren, 2006), also it has great potential uses in industrial processes (Ventosa and Nieto, 1995).

Marine bacteria, especially the benthic microbes could utilize the DNA from dead and degraded organism as sole source of energy. Hence, the attempt to isolate the marine bacteria with potential and unique DNase activity from sediments off Kanyakumari coast, Southern tip of India was initiated. This is a preliminary report on the isolation and characterization of marine bacteria with DNase activity in the largely untapped Kanyakumari coastal areas.

## METHODOLOGY

### Isolation of marine bacteria

Sediment samples were collected from 3 sampling points, off coast Kanyakumari and transferred aseptically to lab. The samples were serially diluted and plated on Zobell marine agar and incubated at room temperature for 48 h. The bacterial isolates were selected at random on the basis of their prominence and distinctness in colony morphology and pigmentation. The isolates were screened for their capacity to produce DNA hydrolyzing activity on DNase test agar plate.

### DNase well diffusion method

The well diffusion method was followed for DNase test employing the standard procedure (Sanchez and Colom, 2010). Petri plates were prepared by pouring 20 ml of DNase test agar and allowed to solidify. The 48 h culture broths inoculated with the bacterial isolates were added to the wells on the agar. After incubation at 37°C for various time points (24 h, 48 h, & 72 h) the zone of inhibition was measured.

### In vitro DNase assay

The activity of DNase was studied by the reaction of cleavage of calf thymus DNA (20µg/ml) in 10mM Tris -HCl buffer (pH 7.5) containing 2.5mM MgCl<sub>2</sub>, 0.1mM CaCl<sub>2</sub>, and DNase (0.1-2U) at 33°C for 1-15 min. The reaction mixture was supplemented with EDTA (final concentration 2mM) and incubated at 65°C for 10 min. The degradation products were analyzed by electrophoresis in 1% agarose gel stained with ethidium bromide.

### DNase Activity Gel System

For substrate gel, the methodology of Shikawa *et al.*, 1994 was followed. In brief, after SDS – PAGE, the gel was washed with 10mM Tris – HCl (pH 7.8) / 5mM mercaptoethanol at 50°C

for 1 h to remove SDS, and then with 10 mM Tris – HCl (pH 7.8), at 4°C overnight. Then the gels were incubated in 10 mM Tris – HCl (pH 7.8), containing 1mM mercaptoethanol and indicated concentration of  $MgCl_2$ ,  $CaCl_2$  at 37°C for 3 h. When the gel was stained with 0.5µg/ml ethidium bromide, apparent endonuclease activity was detected as dark areas on fluorescent background by UV transillumination of the gel.

#### Effect of NaCl concentration on DNase production of potential isolate

The activity of crudes in potential strains KVCMS-6A 20, KVCMS-6A 23, KVCMS-7A 6 and KVCMS-8A 12 in presence of NaCl (2-40%) was measured by standard assay method and DNase activity through well diffusion method.

#### Characterization of isolate

Characterization of the isolate was carried out by traditional biochemical methods and molecular characterization of the 16S rRNA gene of the potential isolate was done through commercial sequencing and the organism after analysis with CLUSTAL X, the phylogenetic relationship was established using phyip and MEGA 4 and submitted to Genbank.

## RESULT AND DISCUSSION

Marine environment is bestowed with high microbial consortium and biologically productive ecosystem inspite of the extreme environmental conditions. To thrive in these extreme conditions of physical and chemical changeovers, the marine bacteria have developed strategies to adapt to this environment. To maintain their structure and physiology, they are compelled to produce specialized metabolites and potent extracellular hydrolytic enzymes to assimilate the nutrients available around them. Although utilization of dissolved proteins has been investigated to some extent (Hollibaugh and Azam,

1983), the mechanism of utilization of dissolved macromolecules in the marine environment has not been extensively studied. Few references are available on the rapid hydrolysis of extracellular DNA in the marine environment by cell associated and extracellular nuclease. In the present work, the bacterial isolates obtained from the sediment samples collected from various depths off coast Kanyakumari was analyzed for their ability to produce extracellular enzyme, DNase.

#### Isolation of marine microbe (s)

In the deep sea environment, sediments form a reservoir of microbes with enzymatic action with potential biotechnological applications. Kaboyashi *et al.*, 2008 reported a number of cultivable aerobic microbes with variety of enzyme activities from the deep sea floor sediment off shore Shimokita Peninsula, Japan. In a view to isolate microbes with novel enzymes activities

**Table 2.** Screening and isolation of extracellular DNase activity by different isolates

No.	Bacterial Isolates	DNase TestZone(Mm) After 72 Hours
1.	KVCMS- 6A 2	23(a)
2.	KVCMS- 6A 3	28(b)
3.	KVCMS- 6A 6	21(a)
4.	KVCMS- 6A 8	24(a)
5.	KVCMS- 6A 9	28(b)
6.	KVCMS- 6A 13	26(b)
7.	KVCMS- 6A 20	34(c)
8.	KVCMS- 6A 21	27(b)
9.	KVCMS- 6A 23	31(c)
10.	KVCMS- 6A 25	27(b)
11.	KVCMS- 6A 31	28(b)
12.	KVCMS- 7A 1	28(b)
13.	KVCMS- 7A 2	24(a)
14.	KVCMS- 7A6	33(c)
15.	KVCMS- 7A 7	24(a)
16.	KVCMS- 7A 17	25(a)
17.	KVCMS-7A 18	25(a)
18.	KVCMS- 7A 19	28(b)
19.	KVCMS- 7A 25	25(a)
20.	KVCMS- 8A 2	20(a)
21.	KVCMS- 8A1 2	35(c)
22.	KVCMS- 8A13	27(b)
23.	KVCMS- 8A15	25(a)

Values are mean of triplicate experiments Duccants multiple analysis were carried out.

a=20-25mm, b=26-30mm, c=>30mm

**Table 1.** Isolation of marine microbes

Sample No	Number of Isolates Obtained	Number Of Isolates Positive For Dnase
6A	41	11
7A	43	8
8A	27	4
total	111	23

in peninsular India, sediment samples at 3 different sampling points off coast Kanyakumari were collected and transported aseptically to the laboratory and labeled as 6A, 7A, and 8A respectively. The collected samples were serially diluted, plated on Zobell marine agar and the culturable heterotrophic aerobic bacteria were isolated and stored for further studies. Totally, 111 isolates (Table.1) were obtained from the sampling points 6A (41), 7A (43) and 8A (27) respectively. The isolated strains were screened for DNase activity by streaking onto a DNase test agar plate. Out of 111 isolates, 23 isolates which showed clearing zone around the streak with pink halos on DNase test agar were selected as DNase producing isolates and further to pick up the best strain, well diffusion method was employed.

**Table 3.** DNase production of selected bacterial isolates on DNase test agar

Bacterial Isolates	DNase Test Zone diameter in mm		
	24hrs	48hrs	72hrs
KVCMST 6A 20	14	26	34
KVCMST 6A 23	16	25	31
KVCMST 7A 6	16	26	33
KVCMST 8A 12	20	28	35

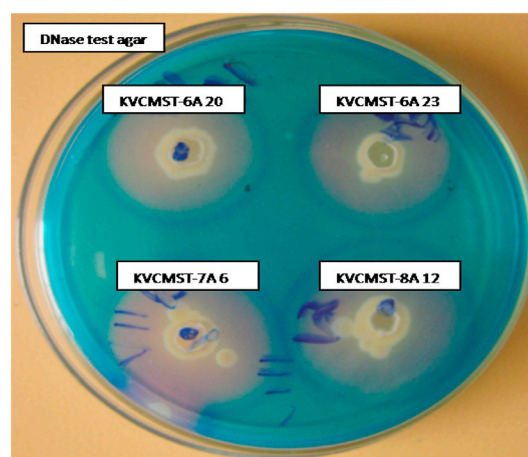
Values are observation of mean  $\pm$  triplicate experiments

**Table 4.** DNase production for KVCMST-6A20 at various NaCl concentrations on DNase test agar

NaCl concn. (%)	DNase test Zone diameter in mm			Optical Density (600nm) at 24 hrs
	24hrs	48hrs	72hrs	
2	14	26	34	0.832
4	12	20	22	0.744
6	10	19	21	0.547
8	10	19	21	0.421
10	8	18	20	0.156
12	8	18	20	0.143
14	8	18	20	0.133
16	6	14	16	0.127
18	6	14	16	0.105
20	4	10	12	0.085
22	4	10	12	0.067
24	4	10	12	0.043

### Screening for extracellular DNase

The culture filtrate of bacterial isolates positive on DNase test agar were analyzed for extracellular DNase production employing agar well diffusion method. Although, all the 23 isolates demonstrated extracellular DNase activity, 2 strains from sampling point 6A designated as KVCMST-6A 20, KVCMST-6A 23, one strain from sampling point 7A denoted by KVCMST-7A 6, and yet another strain from sampling point 8A named KVCMST-8A 12 were shown to produce maximum DNase activity and these strains were used for further studies (Table. 2). DNase test



**Fig. 1.** Extracellular DNase activity of the selected isolates on DNase test agar with toluidine blue.

**Table 5.** DNase production for KVCMST-6A23 at various NaCl concentrations on DNase test agar

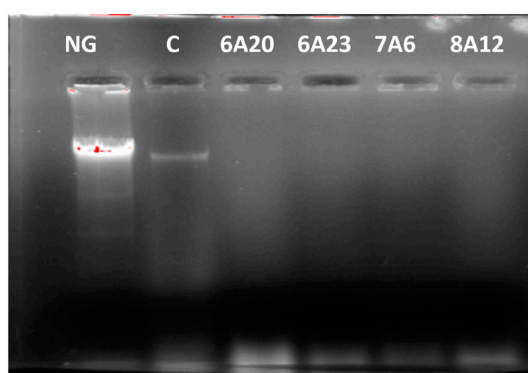
NaCl concn. (%)	DNase test Zone diameter in mm			Optical Density (600nm) at 24 hrs
	24hrs	48hrs	72hrs	
2	16	25	31	0.891
4	12	18	20	0.369
6	12	18	20	0.265
8	10	12	18	0.243
10	10	12	18	0.214
12	10	12	18	0.207
14	8	11	13	0.193
16	8	11	13	0.187
18	6	8	10	0.057
20	6	8	10	0.044
22	6	8	10	0.026
24	6	8	10	0.015

agar is generally used for screening of microbes for DNase production. A thermophilic fungus was isolated and found to produce extracellular DNase employing this method (Landry *et al.*, 2014).

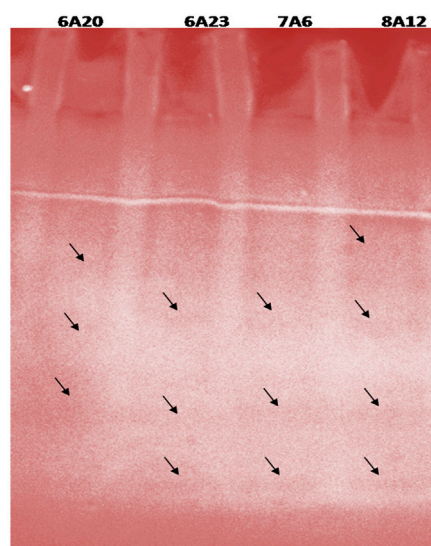
#### Time – Course study for DNase production

Based on the preliminary screening, four strains that produce maximum zone of > 30mm on 72 hours (Fig. 1) were further analyzed for production of extracellular deoxyribonuclease activity at different time points on DNase test agar employing agar well diffusion method. Test agar plates were incubated 72 h to analyze the production of DNase and the zone measured at 24, 48, and 72 hours. The zone of DNase activity

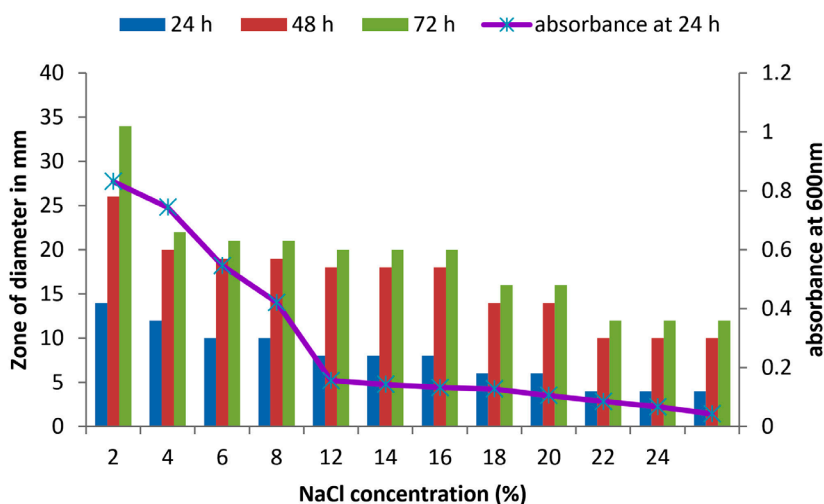
by the strains KVCMST-6A 20, KVCMST-6A 23, KVCMST-7A 6 and KVCMST-8A 12 were shown in Table. 3. Among the four strains, KVCMST-8A 12 demonstrated a prominent zone right from 24 hours up to 72 hours compared to other strains. However, the other three strains KVCMST-6A 20, KVCMST-6A 23 and KVCMST-7A 6 were not to be excluded as they produce a zone on par with KVCMST-8A 12.



**Fig. 2.** Degradation of prokaryotic DNA demonstrated by in vitro DNase activity on agarose gel by the selected bacterial isolates. NG- negative control, C- control DNA



**Fig. 3.** Activity gel analysis of DNase activity exhibited by the crude extracts of selected bacterial isolates

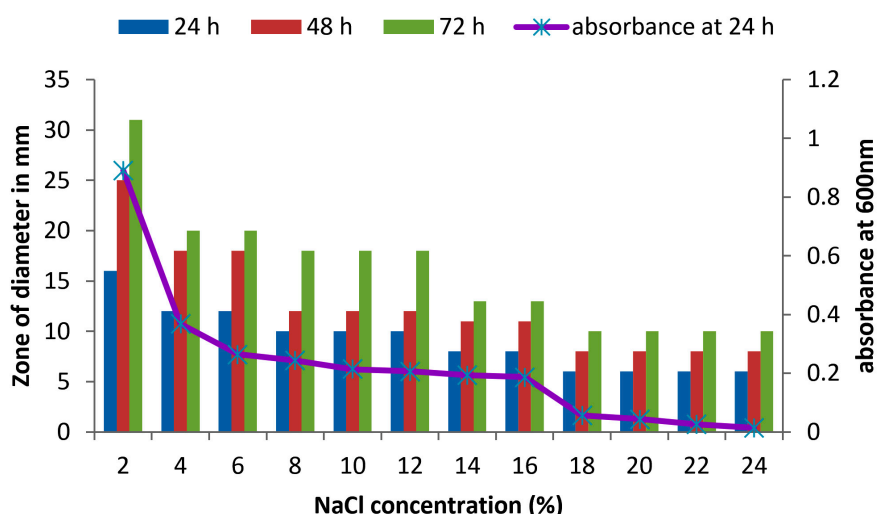


**Fig. 4.** Effect of NaCl on growth and DNase production of KVCMST-6A20. The zone of clearance in DNase test agar at various time of incubation indicated by bars: 24 hours, 48 hours, 72 hours and its growth indicated by its absorbance at 24 hours.

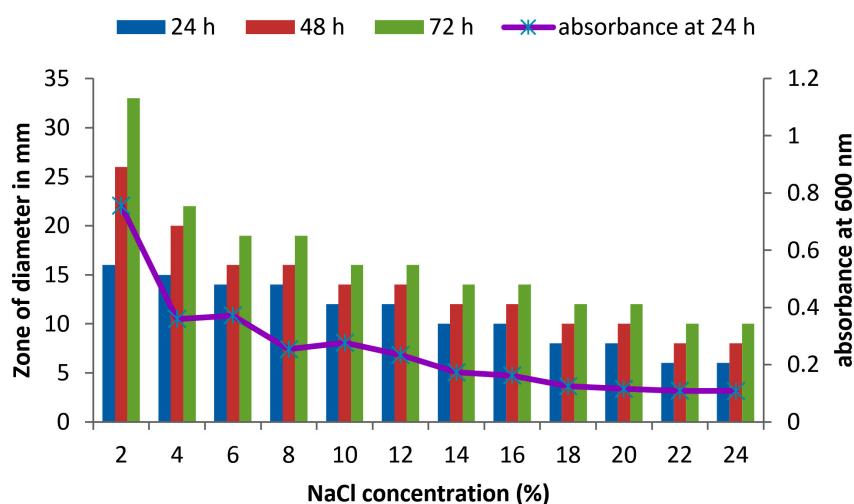
### *In vitro* DNase activity

DNA cleavage is one of the important mechanism to arrest the growth of bacteria and viruses, in control of diseases particularly cancer, there is a considerable interest in development of enzymes suitable to cleave DNA. The selected strains KVCMS-6A 20, KVCMS-6A 23, KVCMS-7A 6, and KVCMS-8A 12 were subjected to DNA cleavage assay on prokaryotic bacterial

DNA to demonstrate the *in vitro* DNase activity employing electrophoretic technique. When the crude extracts of the four isolates were incubated with DNA in tris buffer for 30 minutes and electrophoresed on 0.8% agarose, there was clear cut degradation of DNA (Fig. 2, lane 3 to 6) compared to the control DNA (lane 2) and extract without sample DNA (lane 1). The DNA cleavage potential of the isolates was evidenced



**Fig. 5.** Effect of NaCl on growth and DNase production of KVCMS-6A23. The zone of clearance in DNase test agar at various time of incubation indicated by bars: 24 hours, 48 hours, 72 hours and its growth indicated by its absorbance at 24 hours.



**Fig. 6.** Effect of NaCl on growth and DNase production of KVCMS-7A6. The zone of clearance in DNase test agar at various time of incubation indicated by bars: 24 hours, 48 hours, 72 hours and its growth indicated by its absorbance at 24 hours.



by the complete disappearance of DNA. Song and Zhang (2008) have reported a novel – nonspecific nuclease from thermophilic bacteriophage. However, optimum rate of conditions like substrate concentration, enzyme activity, enzyme concentration, specificity, temperature, incubation period, pH, effect of cations and inhibitors need to be carried out.

#### Substrate gel analysis of DNase

For demonstration of *in vitro* enzyme activities, substrate gel substrate system plays a vital role. It helps not only to locate the presence

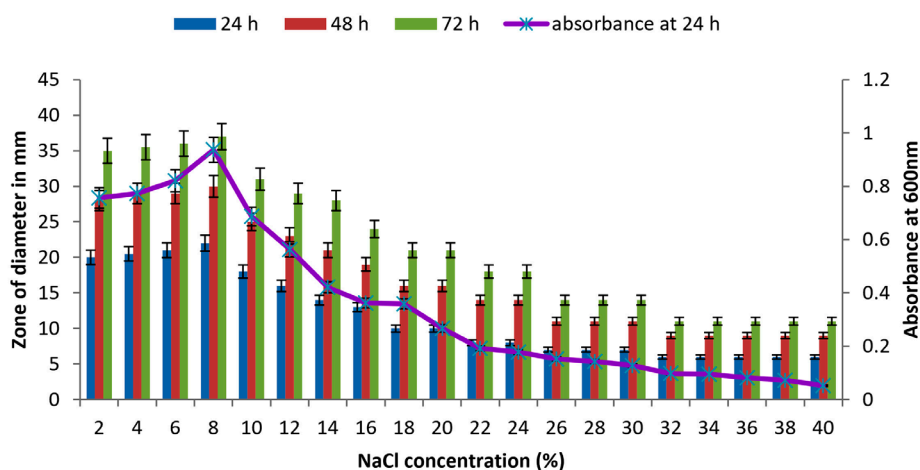
of enzyme, but also indicates whether the enzyme is in active state, thus to isolate and purify this enzyme, substrate gel analysis was carried out. In activity gel analysis, the crude extracts of the

**Table 7.** DNase production for KVCMST-8A12 at various NaCl concentrations on DNase test agar and Effect of NaCl on growth of KVCMST-8A12

NaCl concn. (%)	DNase test Zone diameter in mm			Optical Density (600nm) at 24 hrs
	24hrs	48hrs	72hrs	
2	20	28	35	0.756
4	20.5	29	30.5	0.773
6	21	29	36	0.821
8	22	30	37	0.937
10	18	25	31	0.687
12	16	23	29	0.564
14	14	21	28	0.421
16	13	19	24	0.362
18	10	16	21	0.359
20	10	16	21	0.267
22	8	14	18	0.192
24	8	14	18	0.178
26	7	11	14	0.152
28	7	11	14	0.143
30	7	11	14	0.127
32	6	9	11	0.097
34	6	9	11	0.095
36	6	9	11	0.081
38	6	9	11	0.072
40	6	9	11	0.052

**Table 6.** DNase production for KVCMST-7A6 at various NaCl concentrations on DNase test agar

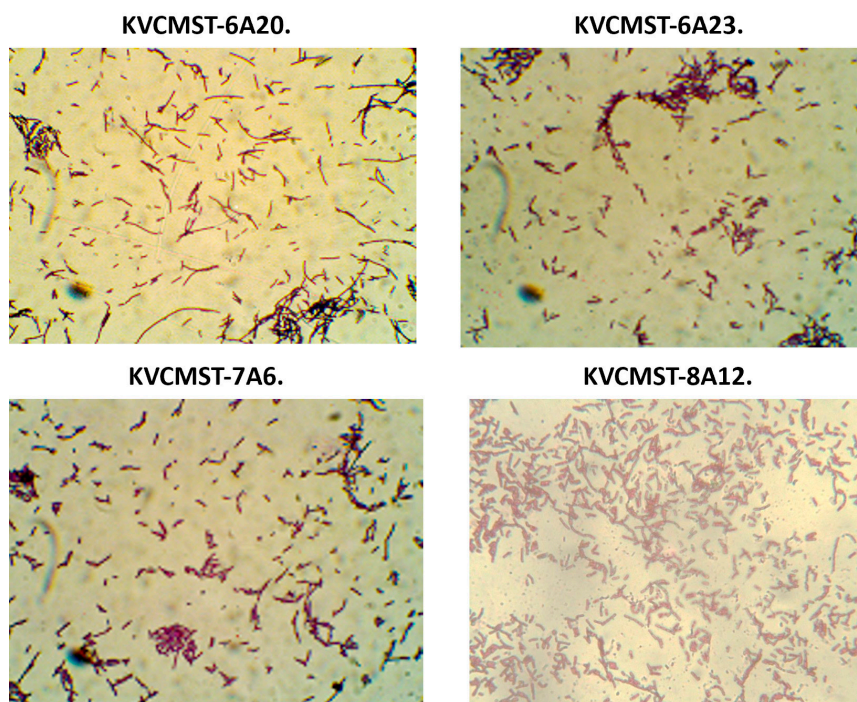
NaCl concn. (%)	DNase test Zone diameter in mm			Optical Density (600nm) at 24 hrs
	24hrs	48hrs	72hrs	
2	16	26	33	0.756
4	15	20	22	0.360
6	14	16	19	0.372
8	14	16	19	0.254
10	12	14	16	0.277
12	12	14	16	0.234
14	10	12	14	0.174
16	10	12	14	0.162
18	8	10	12	0.125
20	8	10	12	0.116
22	6	8	10	0.109
24	6	8	10	0.108



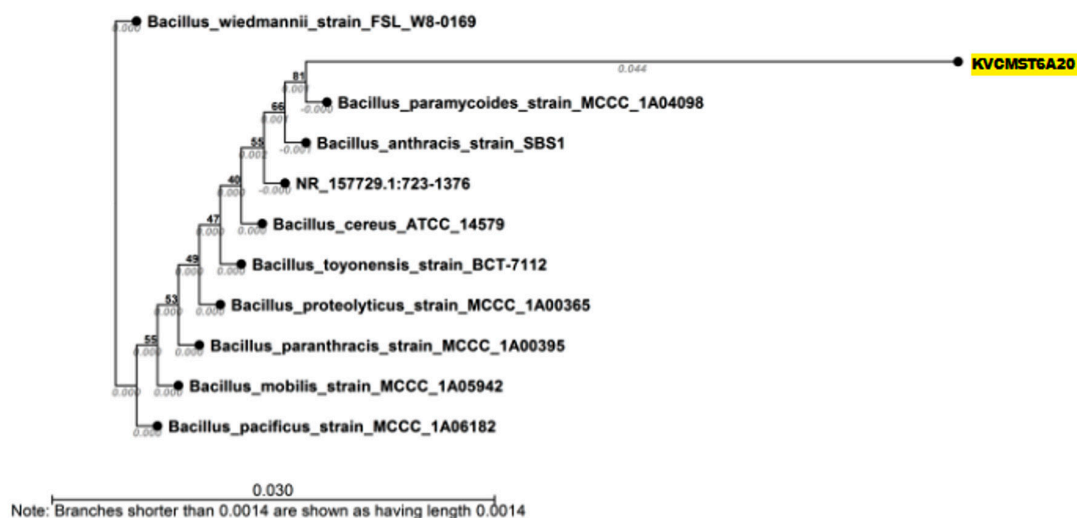
**Fig. 7.** Effect of NaCl on growth and DNase production of KVCMST-8A12. The zone of clearance in DNase test agar at various time of incubation indicated by bars: 24 hours, 48 hours, 72 hours and its growth indicated by its absorbance at 24 hours.

bacterial isolates KVCMS-6A 20, KVCMS-6A 23, KVCMS-7A 6, and KVCMS-8A 12 were separated by SDS polyacrylamide gel in which Calf thymus

DNA was incorporated. After electrophoresis, the gel was washed and incubated in appropriate buffer and DNase activities were revealed by



**Fig. 8.** Gram staining characteristics of the bacterial strains KVCMS-6A 20, KVCMS-6A 23, KVCMS-7A 6 and KVCMS-8A 12.



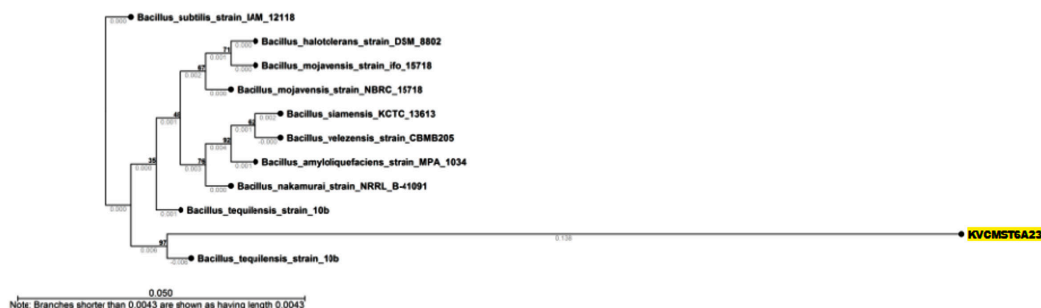
**Fig. 9.** Neighbor joining tree based on 16S rRNA sequences showing the phylogenetic relationship between KVCMS-6A 20 and other related species of the genus *Bacillus* and related reference microorganisms (Accession no MG786828).



**Table 8.** Biochemical Characteristic of the isolates

No.	Phenotypic/ Biochemical	Strains			
		KVCMST 6A 20	KVCMST 6A 23	KVCMST 7A 6	KVCMST 8A 12
1	Grams Reaction	G+ rods	G+ rods	G+ rods	G+ rods
2	Motility	Motile	Motile	Motile	Motile
3	Pigmentation	Colorless	Colorless	Colorless	Colorless
4	Colony Morphology	Large Spherical	Large Spherical	Large Spherical	Large Spherical
5	Spore Formation	+	+	+	+
6	Growth at	4°C	4°C	4°C	4°C
7	Optimum Temperature	37°C	37°C	37°C	37°C
8	Optimum pH	7.0	7.0	7.0	7.0
9	Optimum % NaCl	2%	2%	2%	8%
10	Lactose fermentation on MacKonkey	LF	LF	LF	LF
11	Indole Production	-	-	-	-
12	Methyl Red	-	-	-	-
13	Voges –Prousker	+	+	+	+
14	Citrate Utilization	+	+	+	+
15	Starch Hydrolysis	+	+	+	+
16	Urea Hydrolysis	-	-	-	-
17	TSI Agar test				
	(1) Acid	+	+	+	+
	(2) Alkaline	+	+	+	+
	(3) Gas production	-	-	-	-
	(4) H <sub>2</sub> S	+	+	+	+
18	Catalase	+	+	+	+
19	Oxidase	-	-	-	-
20	Nitrate Reduction	+	+	+	+
21	Casein Hydrolysis	+	+	+	+
22	Gelatin Hydrolysis	-	-	-	-
23	Acid Fast staining	+	+	+	+
24	Utilization of Sugars	+	+	+	+
25	Utilization of Amino acids	+	+	+	+

(+ present / - absent / W- Weak positive)

**Fig. 10.** Neighbor joining tree based on 16S rRNA sequences showing the phylogenetic relationship between KVCMST-6A 23 and other related species of the genus *Bacillus* and related reference microorganisms (Accession no MH938091).

staining with ethidium bromide. The enzyme activity which appeared as dark band (Fig. 3) was shown in arrow marks.

#### Effect of NaCl on growth and DNase production of potential strains

There are only few reports on halophilic nuclease production by halophiles, including *Micrococcus* variants, *Var halophilus* (Kamekura and Onishi 1978) and *Bacillus halophilus* (Onishi et al., 1983, Ventosa 2015). In order to know the halophilic nature and capacity of DNase production of all strains it is necessary to check the growth and DNase production at various NaCl concentrations (Table. 4 to Table. 7). The isolates were grown at various NaCl concentrations and

the overnight grown culture was centrifuged and supernatant was assayed for DNase production on toluidine blue DNA agar, DNA hydrolysis zone were noted. The strains exhibited maximum DNase production and growth at 7.5% except KVCMS-8A 12 it showed maximum DNase production at 8% (1.36M) concentration but the strain was capable of producing DNase upto 40% NaCl concentration tested (6.81M) (Fig.4). Onishi et al., 1983 identified a moderate halophile, *Bacillus* sp N23-2 produced an extracellular nuclease during the growth in 1 to 2M NaCl. KVCMS-8A 12 is a true halophilic microbe as it possess extracellular deoxyribonuclease even at 6.81M.

**Table 9.** Nucleotide informations of KVCMS-6A 20

Sequence information		Nucleotide distribution table		
Sequence type	DNA	Nucleotide	Count	Frequency
Length	653bp	Adenine (A)	166	0.254
Name	KVCMS-6A_20	Cytosine (C)	140	0.214
Weight (single-stranded)	202.878 kDa	Guanine (G)	193	0.296
Weight (double-stranded)	403.527 kDa	Thymine (T)	154	0.236
		C + G	333	0.510
		A + T	320	0.490

**Table 10.** Nucleotide informations of KVCMS-6A 23

Sequence information		Nucleotide distribution table		
Sequence type	DNA	Nucleotide	Count	Frequency
Length	625bp	Adenine (A)	171	0.274
Name	KVCMS-6A_23	Cytosine (C)	145	0.232
Weight (single-stranded)	193.956 kDa	Guanine (G)	178	0.285
Weight (double-stranded)	386.23 kDa	Thymine (T)	131	0.210
		C + G	C + G	323
		A + T	A + T	302

**Table 11.** Nucleotide informations of KVCMS-7A 6

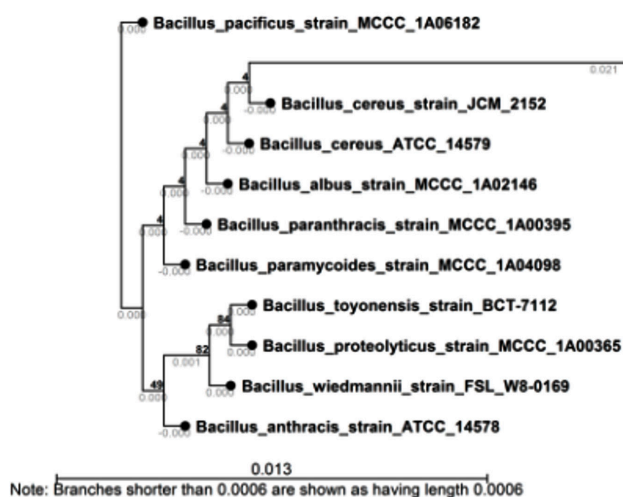
Sequence information		Nucleotide distribution table		
Sequence type	DNA	Nucleotide	Count	Frequency
Length	710bp	Adenine (A)	184	0.259
Name	KVCMS-7A_6	Cytosine (C)	158	0.223
Weight (single-stranded)	220.56 kDa	Guanine (G)	211	0.297
Weight (double-stranded)	438.755 kDa	Thymine (T)	157	0.221
		C + G	C + G	369
		A + T	A + T	341

### Identification of strain

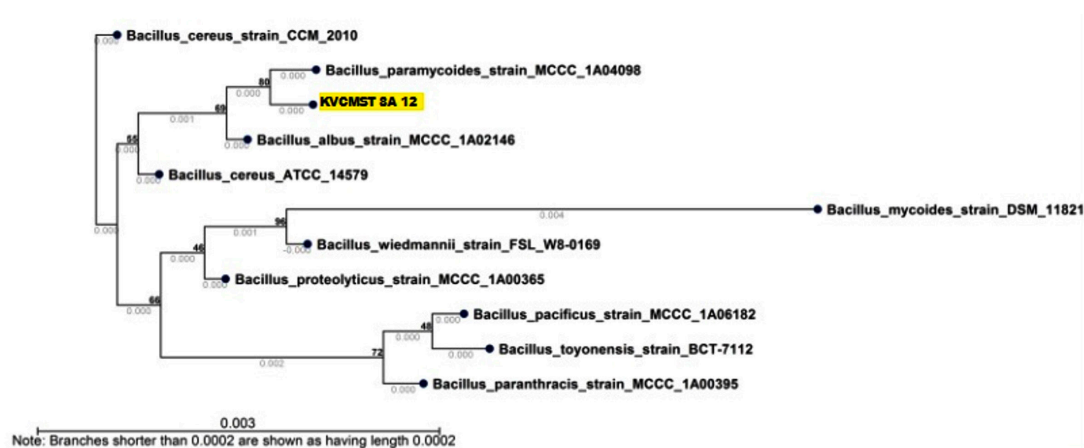
Having known that the crude extracts from the bacterial isolates were (KVCMS-6A 20, KVCMS-6A 23, KVCMS-7A 6 and KVCMS-8A 12) capable of producing extra cellular DNase and its *in vitro* DNase activity have been demonstrated both in *in vitro* DNase activity on agarose gel and activity gel analysis on SDS-PAGE, it is necessary that the taxonomical identity of the bacterial isolates have to be established. Therefore, employing various biochemical tests (Table.3 and Table.4), these isolates were tentatively identified as *Bacillus* Sp

belonging to *Bacillus cereus* group. They were gram positive (Fig.8) anaerobic, spore forming, rod shaped organisms with smooth, convex, round, glossy and white colony morphology. From which KVCMS-8A 12 tolerated up to 40% NaCl and growth occurs on Mac Conkey agar. All strains showed positive for hydrolysis of starch, casein and DNA, but negative for hydrolysis of gelatin, urease, indole and negative for oxidase.

On the basis of phenotypic characteristics and the comparison of partial 16S rRNA gene sequences, all the isolates KVCMS-6A 20, KVCMS-



**Fig. 11.** Neighbor joining tree based on 16srRNA sequences showing the phylogenetic relationship between KVCMS-7A 6 and other related species of the genus *Bacillus* and related reference microorganisms (Accession no MG786829).



**Fig. 12.** Neighbor joining tree based on 16srRNA sequences showing the phylogenetic relationship between KVCMS-8A 12 and other related species of the genus *Bacillus* and related reference microorganisms (Accession no MG786830).

**Table 12.** Nucleotide informations of KVCMS-8A 12

Sequence information		Nucleotide distribution table		
Sequence type	DNA	Nucleotide	Count	Frequency
Length	1,557bp	Adenine (A)	394	0.253
Name	KVCMS-8A_12	Cytosine (C)	354	0.227
Weight (single-stranded)	483.84 kDa	Guanine (G)	478	0.307
Weight (double-stranded)	962.15 kDa	Thymine (T)	331	0.213
		C + G	C + G	832
		A + T	A + T	725

6A 23, KVCMS-7A 6 and KVCMS-8A 12 were identified as member of the *Bacillus cereus* family (accession no MG786828, MH938091, MG786829, MG786830 respectively) (Fig.9 to Fig.12). *Bacillus cereus* group comprises 11 closely related species, including *B. anthracis*, along with *B. cereus*, *B. thuringiensis*, *B. mycoides*, *B. weihenstephanensis*, *B. pseudomycoides* and the recently identified *B. gaemokensis*, *B. manliponensis*, *B. cytotoxicus*, *B. toyonensis*, *B. bingmayongensis* (Liu *et al.*, 2017). Rohban *et al.*, (2009) have isolated hydrolyzing enzymes belonging to the members of genera such as *Bacillus* and *Halobacillus*. In their study most of the DNase produces were of the genera *Halomonas*, however in our study, the best DNase producing isolate was found to be *Bacillus* Sp.

In conclusion, the strains KVCMS-6A 20, KVCMS-6A 23, KVCMS-7A 6 and KVCMS-8A 12 isolated from the sediment sample collected at a depth of 26.4 m off Kanyakumari coast possess a strong DNase activity as evidenced by the DNase test agar, *in vitro* DNase assay and gel activity system and with the help of phylogenetic analysis all strains were found to belong to *Bacillus cereus* family. Among which the strain KVCMS-8A 12 can be regarded as a halophilic, valuable source of the enzyme DNase and demands the purification and characterization of this enzyme which can be used in various biotechnological and biomedical fields. In our present study, we were able to demonstrate a zone greater than the purified enzyme as well as to that of the results obtained by Kample *et al.*, (2011) by employing only the crude culture supernatant of KVCMS-8A 12. This indicates a positive approach that this enzyme can be purified in future and this isolate

would promise a valuable source of the enzyme DNase for commercial exploitation.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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#### AUTHORS' CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

#### DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

#### ETHICS STATEMENT

Not applicable.

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