OPTIMIZATION OF PH AND TEMPERATURE FOR DEOXYRIBONUCLEASE PRODUCING BACTERIA OBTAINED FROM SOIL

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ABSTRACT

The source of DNA for Deoxyribonuclease (DNase) producing bacteria in soil is the decaying plant cells, dead animal and bial cells. Very few organisms produce DNase extracellularly. Soil samples from different areas of Akola district (India) were screened for deoxyribonuclease production. Of the 100 soil samples screened; 10 efficient deoxyribonuclease producing bacteria were selected for morphological and biochemical characterization. These cultures were grown in nutrient broth containing DNA at various pH and temperature. The pH chosen were 3,5,7,9 and 10 and temperatures chosen were 10°C, 20°C, 30°C, 40°C and 50°C. The broth was centrifuged to separate out the cells from the above mentioned tubes. The supernatant was taken as a source of deoxyribonuclease enzyme. Each pH and temperature tube was then assayed for DNA degradation. Tubes showing maximum amount of DNA degradation in the respective pH and temperature tube was chosen as the optimum pH and temperature. In order to maximize the enzyme concentration it is necessary to optimize the conditions around cell.

Keywords: Soil, deoxyribonuclease, bacteria, pH, optimization, temperature, assay.

Abbreviations used: DNA, Deoxyribonucleic acid; DNase, Deoxyribonuclease; SN, *Staphyloccal* nuclease; 8-OHGua, 8-hydroxyguanine; ROS, reactive oxygen species

INTRODUCTION

Deoxyribonucleases are group of enzymes that are capable of hydrolyzing the phosphodiester linkages of nucleic acids. Nucleases play vital role in cellular functions; specifically 4 R's i.e. replication, repair, restriction and recombination. These are also involved in transposition, transcription and topoisomerization, as well as RNA processing, RNA splicing, editing and interference (Mishra 2002). DNases has wide range of applications both inside and outside the cells. By using nucleases in different ways, it has become easier to recombine DNA, remove harmful genes, and replace single gene on DNA strand; applications include gene therapy for genetic diseases, genetic engineering, DNases has been used in chemotherapeutic and industrial fields also.

The accumulation of 8-OHGua induces G: C to T: A transversion during DNA replication that is related to mutagenic events. The 8-OHGua is formed by ROS which is removed from DNA by endonucleases in DNA salvage pathways (Hui *et al.* 2003). *Phytophthora sojae* and *Phytophthora ramorum* the devastating pathogen of soybean and oak and other *Phytophthora sp* were studied by TILLING (Targeting Induced Local Lesions In Genomes); one of the method of reverse genetics in which single strand specific endonuclease have been exploited (Tierney *et al.* 2005). In DNA footprinting also DNases are used which is a method of investigating the sequence specificity of DNA-binding proteins. This technique is used to study protein-DNA interactions both outside and within cells (www.wikipedia.org). DNases cleaves chromosomal DNA into nucleosomal fragments in 180-200 bp in the process of apoptosis and later on these fragments are broken into 20-30 nucleotides and then into single nucleotides (Sluyser Mels 2005). Qin *et al.* (2005) has explored the feasibility of using capsid-targeted viral inactivation (CTVI) as an antiviral strategy against dengue infection, and has constructed a plasmid expressing a fusion protein consisting of SN fused to dengue 2 virus capsid protein (D2C) and investigated its effects on the production of infectious virions when introduced into baby hamster kidney (BHK) cells infected with dengue virus. The results indicated that D2C-SN was expressed and tolerated in this mammalian cell culture. The products of nucleases i.e. nucleotides and nucleosides are found to be antiviral. Derivatives of these products are prepared which mimics the viral activities (Howell *et. al.* 1988).

Nucleases have been studied from plants also. The fruit bodies of *Lentinus edodes* produce two acid nucleases, nucleases Le1 and Le3; both are candidates for the enzymes producing a tasty substance, 5'-GMP. To obtain the basic information on the mechanism of production of 5'-GMP, and structure-function relationship of these nucleases, the primary structure of nuclease Le1 was estimated by both protein chemistry and gene cloning (Kobayashi *et al.* 2000). Ribonuclease II was isolated from the somes of corn roots, and was partially purified by gel filtration. It has a pH optimum

plateau from 5.4 to 7.0.Nuclease was isolated from the large particles of a corn root homogenate. Nuclease I hydrolyzes both RNA and DNA. It has a pH optimum at 6.2 and a molecular weight of 31,000 (Curtis 1968). Incubation of barley (*Hordeum vulgare*) half-seeds with gibberellic acid enhances the secretion of ribonuclease and deoxyribonuclease from aleurone tissue. Both activities had a pH optimum of 6.0 and a temperature optimum of 55°C. When the medium from gibberellic acid-treated half-seeds was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, the major ribonuclease and deoxyribonuclease activity bands comigrated (Peter *et al.* 1998). Two RNases, wheat leaves designated WL-RNase I and II, were separated from extracts of senescing wheat leaves. WL-RNase II, but not WL-RNase I, was inhibited by EDTA. A period of time was necessary for the full effect of EDTA to be observed. Zinc ions abolished the EDTA effect (Sodek *et al.*1970). The absence of sugar or phosphate in culture medium has marked effect on the activity of RNases and nucleases expressed in maize endosperm suspension cells. Withdrawal of sugar from the culture medium resulted in a substantial increase in RNase and nuclease activities, whereas deprivation of phosphate during the same period of growth had no detectable effect on either of these activities (Daniel *et. al.* 2002). Ribonuclease is also purified from cowpea cotyledons (Eneas *et. al.* 1994).

Nucleases have also been studied from insects also. A nuclease activity having a pH optimum of 10.5 was identified in the midgut contents of the larvae of *Spodoptera litura* with optimum activity at 55°C (Ahmad *et. al.*, 1983). The plasma membrane of protozoan parasites has been the subject of intense research interest because it serves as the interface between the parasite and its host. Nucleases are also present in the plasma membrane of protozoan parasites (Gottlieb M., 1989). The characteristics and mode of action of a single-strand-specific nuclease isolated from rat liver endoplasmic reticulum are investigated with respect to its DNA and RNA substrates. The enzyme exhibits small pH dependence between pH 6–9 and maximum activity is observed at pH 7–7.5 for both DNase and RNase activities (Sofia *et. al.* 1987).

bial nucleases have been preferred over others because of their ease in isolation. coccal nuclease has been studied in detail (Anwar *et. al.* 1996). An extracellular nuclease from *Rhizopus stolonifer* (designated as nuclease Rsn) was purified. The optimum pH of the enzyme was 7.0 and was not influenced by the type of metal ion used. The optimum temperature of the enzyme for DNA hydrolysis was found to be 45°C in presence of metal ions (Rangrajan *et. al.* 1999). A single strand specific and guanylic acid – preferential extracellular DNase has been purified from *Basidiobolus haptosporus* (Desai *et. al.* 2000). DNase has also been isolated from *Penicillium citrinum* (Ying *et al*. 2006), *Streptomyces thermonitrificans* (Deshmukh *et al.* 2004) and *Mycobacterium tuberculosis* (Verma *et. al.* 2005).

MATERIALS AND METHODS

Collection of soil samples from different areas of Akola (India) up to a specified depth (Gary *et al.* 2006), screening of nuclease producing bacteria on DNase test agar containing toludiene blue (Schreier, 1969) and DNase test agar containing methyl green (Smith *et al.* 1977). Morphological and biochemical characterization of efficient cultures was performed. Optimization of p^H and temperature was performed by Metachromatic Agar Well Diffusion Technique (Lachica *et al.* 1972).

1. Soil Sample Collection:

Soil samples were collected from surrounding villages of Akola District. Soil samples were collected at specified depths up to 10 cm using a 2.54-cm-diameter soil corer. The corer was cleaned between samples with water followed by methanol and samples were placed in zip-lock plastic bags and stored for no longer than 7 days at 6°C. (Gary *et al.* 2006).

2. Screening of DNase Producers:

These soil samples were diluted by serial dilution techniques. After colony count 10^{-7} dilution was selected. From all soil samples 0.1 ml. was inoculated on DNase test agar containing toludiene blue and DNase test agar with methyl green. Hydrolysis of DNA on DNase test agar containing toludiene blue is observed as faint pink halos from initial blue color around the colonies because of metachromatic property of toludiene blue. Where as that on DNase test agar with methyl green the hydrolyzed area becomes colorless from green. Toludiene blue on reaction with DNA forms blue color and that with methyl green forms green color. Clear results were obtained in both media. About 100 soil samples were screened and 10 efficient cultures in terms of larger zones of DNA hydrolysis were selected for morphological and biochemical characterization. Selective media were also used for identification of the isolates.

3. Assay of standard deoxyribonuclease:

Because of ease of operation, rapid, convenient and in accordance with objectives of the study; metachromatic agar well diffusion (MAD) technique (Lachica *et al.* 1972) was preferred over other methods. Minor modifications were made in MAD technique in that method was performed in petri dishes instead of plastic plates. Wells were cut on toludiene blue DNA agar (Not DNase test agar with toludiene blue) with a sterile cork borer. The size of cork borer was 2 mm in diameter.

A measured quantity (3) of standard DNase was added by pipette aseptically. As mentioned earlier toludiene blue on reaction with DNA gives blue color. When DNA is hydrolyzed the area around the

wells becomes faint pink and sometimes colorless also because of metachromatic property of toludiene blue. Concentrations of standard DNase were prepared ranging from 1 g/ml to 10 g/ml. These were then added in the wells prepared. Smaller DNase conc. formed smaller zones and higher DNase conc. formed larger zones. Plates were incubated at 30°C for 3 hrs and corresponding zones were noted. A graph was plotted by taking standard DNase on X-axis and zone of DNA hydrolysis on Y-axis.

4. Optimization of pH and temperature:

The isolates were obtained from the areas as Hingna, Chauhatta Bazar, Danori, Gandhigram, Sukoda Phata, Nimbhora, Akot, Tamaswadi, Dahihanda, and Palsod. The isolates were grown in nutrient broth containing 0.2% DNA at various pH as 5, 7, 9, 10 and 11.The pH was adjusted with HCl and NaOH. Same cultures were also grown in nutrient broth containing 0.2% DNA and were incubated at various temperatures as 10°C, 20°C, 30°C 40°C and 50°C. After incubation for 24 hrs at various temperature and pH; the broth tubes were centrifuged at 13,000g for 30 min. to separate out the cells. Being an extracellular enzyme; supernatant was used a source of DNase. This was assayed for DNA hydrolysis.

RESULTS AND DISCUSSION

After repeated screening on DNase test agar with toludiene blue (Plate NO. 1) and methyl green (Plate NO. 2); the DNase producers were taken for biochemical and morphological characterization (Table No. 1). Isolates identified in this manner were also streaked on selective media (Plate NO.3 to 8). Assay of standard nuclease was performed (Plate NO. 9 to 10 and Table No. 2). A standard graph was plotted by taking conc. of DNA on X- axis v/s zone of DNA hydrolysis on Y- axis (Graph 1). Identified isolates grown at various pH and temperature for 24 hrs were centrifuged and supernatant was assayed for DNase concentration on toludiene blue DNA agar (Plate NO.11). DNA hydrolysis zones were noted at various pH and temperature and corresponding concentration according to standard graph were calculated (Table No. 3 and 4). More the amount of DNase produced at particular pH and temp; optimum is the pH and temp for DNase production. Likewise each isolate was assayed for DNase production in pH 3, 5, 7, 9, 10 and at temperature 10°C, 20°C, 30°C 40°C and 50°C. Values in bold in table no. 3 and 4 indicate optimum pH and temperature respectively.

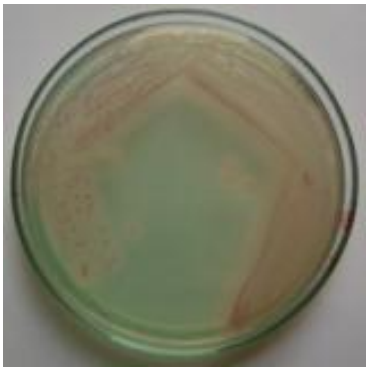

Plate 1: *Serratia marcescens* on DNase test DNase Test agar with methyl green.

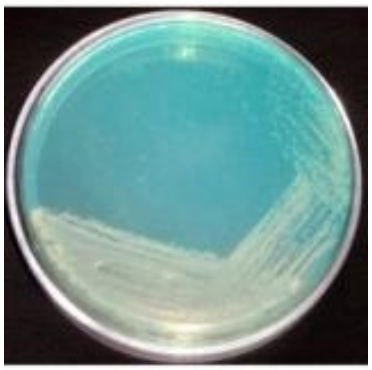

Plate 2: *Staphylococcus aureus* on DNase Test agar with toludiene blue.

Screening of DNase producing bacteria on DNase Test Agar with Toludiene Blue/ Methyl Green.

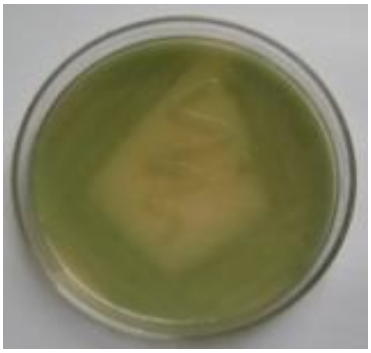

Plate 3: *Pseudomonas sp.* on Cetrimide Agar

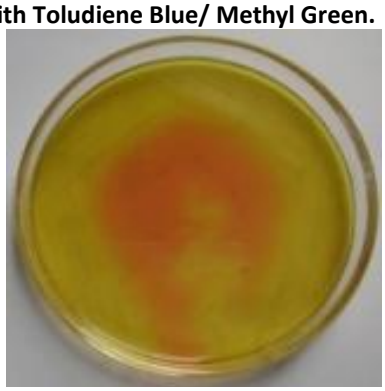

Plate 4: *Staphylococcus sp.* on Manitol Salt Agar

Plate 5: *coccus sp*. on Ammonium Phosphate Agar

Plate 6: *Bacillus sp*. on Starch Agar

Table No. 1: IMViC Reactions

Soil Samples	Gram's nature	Motility	Indole	MR	VP	Citrate	Acid/ Gas	Glucose	Lactose	Mannitol	Bacteria
Hingna	Gram +ve (short rods)	Motile	-ve	+ve	+ve	-ve	Acid Gas	+ve -ve	+ve -ve	+ve +ve	*Bacillus sp.*
Chauhatta Bazar	Gram +ve (short rods)	Motile	-ve	+ve	+ve	-ve	Acid Gas	+ve -ve	+ve -ve	+ve +ve	*Bacillus sp.*
Danori	Gram +ve (cocci in chain)	Non-motile	-ve	+ve	-ve	-ve	Acid Gas	+ve -ve	+ve -ve	+ve -ve	*Streptococcus sp.*
Gandhigram	Gram –ve (short rods)	Motile	-ve	-ve	-ve	+ve	Acid Gas	+ve -ve	-ve -ve	-ve -ve	*Pseudomonas aeruginosa*
Sukoda Phata	Gram +ve (cocci in cluster)	Non-motile	-ve	+ve	-ve	+ve	Acid Gas	+ve -ve	+ve -ve	+ve -ve	*S.aureus*
Nimbhora	Gram –ve (short rods)	Motile	-ve	-ve	-ve	+ve	Acid Gas	-ve -ve	-ve -ve	-ve -ve	*coccus sp.*
Akot	Gram +ve (cocci in cluster)	Non-motile	-ve	+ve	-ve	+ve	Acid Gas	+ve -ve	+ve -ve	+ve -ve	*S. aureus*
Tamaswadi	Gram –ve (rods)	Motile	+ve	+ve	-ve	-ve	Acid Gas	+ve +ve	+ve +ve	+ve +ve	*Serratia marcescens*
Dahihanda	Gram –ve (short rods)	Motile	-ve	+ve	+ve	-ve	Acid Gas	+ve -ve	+ve -ve	+ve +ve	*Bacillus sp.*
Palsod	Gram +ve (short rods)	Motile	-ve	+ve	+ve	-ve	Acid Gas	+ve -ve	+ve -ve	+ve +ve	*Bacillus sp.*

Table No. 2: Assay of Standard Deoxyribonuclease (Enzyme diluted 1:10)

Sr. No.	Conc. of Standard DNase (microg/ml)	Zone of DNA Hydrolysis (mm)
1.	01	2.5
2.	02	04
3.	03	06
4.	04	08
5.	05	10
6.	06	12
7.	07	14
8.	08	16
9.	09	18
10.	10	20

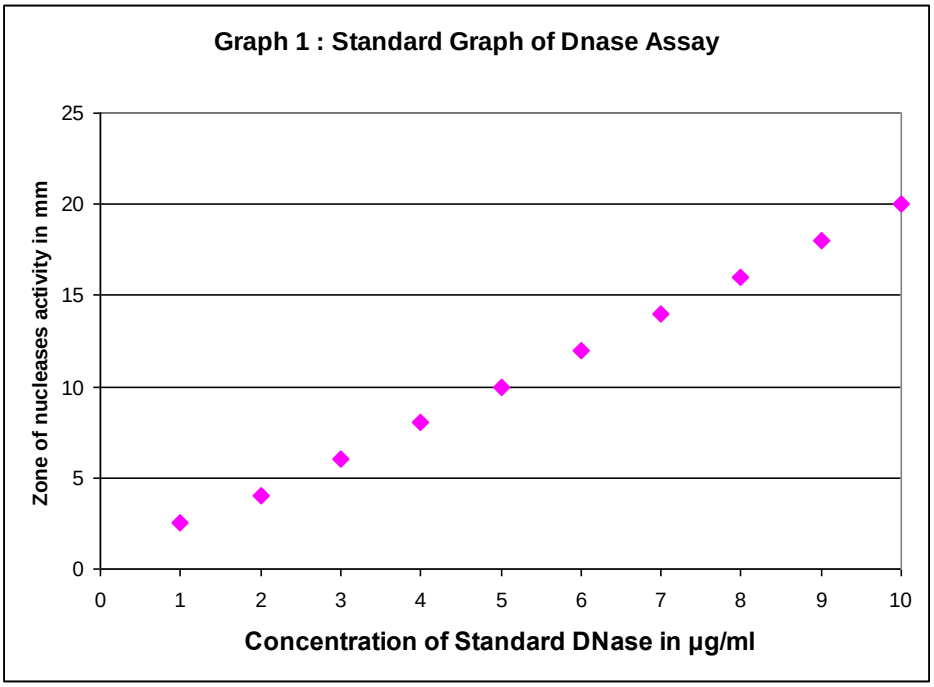

Plate 7: *Bacillus sp* on Starch Agar

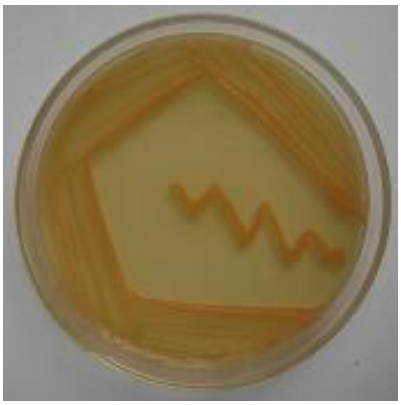

Plate 8: *Serratia marcescens* on Nutrient Agar

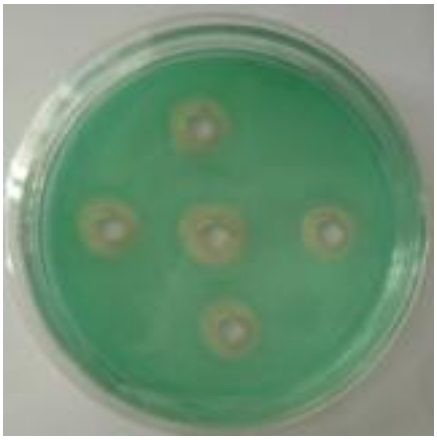

Plate: 9 and 10 Metachromatic Agar Diffusion Assay of std. DNase

Table No.3 Optimization of pH

Sr. No.	Name of area	Bacteria identified	Zone of DNA Hydrolysis (mm)diff. pH values					Corresponding concentration of DNase in microg/ml				
			pH 3	pH 5	pH 7	pH 9	pH 10	pH 3	pH 5	pH 7	pH 9	pH 10
1.	Hingna	*Bacillus sp.*	--	--	**12.2**	4.4	--	--	--	**6.4**	2.2	--
2.	Chauhatta Bazar	*Bacillus sp.*	--	--	**11.6**	4.8	--	--	--	**5.8**	2.4	--
3.	Danori	*Streptococcus sp.*	--	9.8	**11.2**	--	--	--	4.9	**5.7**	--	--
4.	Gandhigram	*Pseudomonas aeruginosa*	--	5.8	**12.2**	--	--	--	2.9	**6.4**	--	--
5.	Sukoda Phata	*S.aureus*	--	4.8	**13.2**	7.0	--	--	2.4	**6.6**	3.5	--
6.	Nimbhora	*coccus sp.*	--	--	**--**	**13.8**	11.2	--	--	4.5	**6.8**	5.7
7.	Akot	*S. aureus*	--	2.8	**13**	--	--	--	1.4	**6.5**	--	--
8.	Tamaswadi	*Serratia marcescens*	--	--	**11**	9.6	3.2	--	--	**5.5**	4.8	1.6
9.	Dahihanda	*Bacillus sp.*	--		5.6	**8.8**	--	--		2.8	**4.4**	--
10.	Palsod	*Bacillus sp.*	--	--	**12.2**	8.4	--	--	--	**6.1**	4.2	--

Table No. 4 Optimization of Temperature

Sr. No.	Name of area	Bacteria identified	Zone of DNA Hydrolysis (mm) at different temperatures.					Corresponding concentration of DNase in microg/ml.				
			10°C	20°C	30°C	40°C	50°C	10°C	20°C	30°C	40°C	50° C
1.	Hingna	*Bacillus sp.*	3.6	9.4	**12.5**	10.4	6.4	1.8	4.7	**6.3**	5.2	3.2
2.	Chauhatta Bazar	*Bacillus sp.*	4	3.2	8.4	**13.8**	11.5	2.0	1.6	4.2	**6.9**	5.8
3.	Danori	*Streptococcus sp.*	7.2	10.8	9.0	**13.2**	6.2	3.6	5.3	4.5	**6.6**	3.6
4.	Gandhigram	*Pseudomonas aeruginosa*	4.8	6.6	**12.7**	9.2	--	2.4	3.3	**6.4**	4.6	--
5.	Sukoda Phata	*S.aureus*	5.2	8.2	**14**	12.4	10.2	2.6	4.1	**7.0**	6.2	5.1
6.	Nimbhora	*coccus sp.*	7.2	10.2	9.5	**13.8**	--	3.6	5.1	4.8	**6.9**	--
7.	Akot	*S. aureus*	4.8	11.2	**13.6**	11.8	6.2	3.9	5.6	**6.8**	5.9	3.1
8.	Tamaswadi	*Serratia marcescens*	10.2	**12.4**	9.8	2.6	--	5.1	**6.2**	4.9	1.3	--
9.	Dahihanda	*Bacillus sp.*	--	3.2	6.2	**8.4**	--	--	1.6	3.1	**4.2**	--
10.	Palsod	*Bacillus sp.*	4.8	7.8	**12**	11.5	9.4	2.4	3.9	**6.0**	5.8	4.7

In order to obtain more DNase production for various applications as mentioned earlier it is necessary to optimize the conditions around the cells. Regarding optimum pH (Table no.3) only *coccus sp*. was found to be halophilic with maximum DNase production of 6.8 micro g/ml at pH 9 and optimum temperature of 40°C. The results complies with observation of Motoaki *et al.* (1970) where optimum pH 9 and optimum temperature 37°C were observed for DNase production for *coccus sp*. Optimum pH 7 and optimum temperature 20°C for DNase production by *Serratia marcescens* were observed which differs from that observed by Jepsen *et al.* (1987) where optimum temperature of 30°C and pH 8, optimum temperature of 37°C and pH 8 were observed for two strains of *Serratia marcescens.* The variation is due to the site of soil sample collection. *Bacillus sp*. was predominant DNase producer with wide range of pH and temperature. *Bacillus sp*. from Hingna village showed maximum DNase production at pH 7and 30°C. Moderately thermophilic *Bacillus sp.* with maximum DNase production at pH 7 and temperature 40°C was found in Chohatta Bazar village. *Bacillus sp*. from Dahihanda village was found to be halophilic but moderately thermophilic DNase producer with optimum pH 9 and temperature 40°C. Optimum pH 7 and temperature 30°C were observed for *Bacillus sp*. from Hingna village. *Bacillus sp*. from Dahihanda village shows somewhat similar optima with that observed by Hiroshi *et al.* (1983) where pH 8.5 and temperature 40°C was optimum for DNase production by *Bacillus sp*. Locke *et al.* (2004) has observed optimum pH 8 and higher temperature optima for streptococcal nuclease which is somewhat similar to that observed by DNase production by *Streptococcus sp.* with pH 7 and temperature 40°C. Because of differences in soil profiles it is obvious to observe little variation in pH and temperature optima for maximum DNase production among the species also. Kamman *et al.* (2006) has observed optimal pH 10 and temperature 50°C for maximum DNase activity of *S. aureus* nuclease contrasting to this both *S. aureus* has

shown pH 7 and temperature 30°C for maximum nuclease production. Robert *et al.* (1976) has found optimum pH 7 and temperature 37°C for *Pseudomonas sp.* nuclease. *Pseudomonas aeruginosa* from Gandhigram village has shown optimum pH 7 and temperature 37°C for maximum nuclease production.

When effect of pH on DNase production among the isolates was compared it was found that *coccus sp.* has produced larger amount of DNase i.e. 6.8 microg/ml followed by *S. aureus, Bacillus sp, Pseudomonas aeruginosa, Serratia marcescens* and lowest production by *Bacillus sp.* from Dahihanda i.e. 4.4 microg/ml.When effect of temperature on DNase production among the isolates was compared it was found that one of the *S. aureus* species produced maximum amount of DNase i.e. 7.0 microg/ml followed by *coccus sp., S. aureus, Bacillus sp, Pseudomonas aeruginosa, Serratia marcescens, Bacillus sp.*. Lowest DNase production was found in the case of *Bacillus sp.* from Dahihanda i.e. 4.2 microg/ml. However all the cultures produced DNase even at 10°C but concentration of DNase was very low. Of the pH and temperature; later has more effect on DNase production. As the soil profile differs optimum conditions also differs within genus also.

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LITERATURE CITED

Ahmad NS and Hadi SM. 1983. Purification and properties of a glycoprotein alkaline nuclease from the larvae of the army worm *Spodoptera litura*. *Insect Biochemistry.* **13** (4): 391- 401.

Anwar QH Subramonian and Puranik VK. 1996: An improved method to distinguish coccal nuclease sensitivity of chromatin. *Journal of Biochemical and Biophysical Method.***33** (1): 59-64.

Curtis M W. 1968. Plant Nucleases I Separation and Purification of Two Ribonucleases and One Nuclease from Corn; *Plant Physiol.* **43:**1332–1338.

Daniel RG, Su-Chih C and Todd EY. 2002. Induction of RNase and nuclease activity in cultured maize endosperm cells following sucrose starvation. *Plant Cell, Tissue and Organ Culture* Volume **68**(2): 163-170.

Desai NA and Vepatu S. 2000. Purification and characterization of the single strand specific and guanylic acid–preferential deoxyribose nuclease activity of the extracellular nuclease from *Basidiobolus haptosporus*. *Eur. J. Biochem.* **267** 5123-5135.

Deshmukh SS and Vepatu S. 2004. Extracellular nuclease from a thermophile, *Streptomyces thermonitrificans*, purification and characterization of the deoxyribonuclease activity. *Federation of European bial Society*. **237** (2): 273-278.

Eneas GF, Jose TP and Joaquim E. 1994. Properties of a partially purified ribonuclease from cowpea cotyledons; *Revista Brasileira de Fisiologia Vegetal.* **6:** 27-31.

Gary MB, Gerald WW, Karen PD, Mark DA, Ann CK, Stephen MG and Jeffrey JS. 2006. Fatty Acid Methyl Ester Analysis to Identify Sources of Soil in Surface Water. *J. Environ Qual* **35**:133-140

Gottlieb M. 1989. The surface membrane 3′-nucleotidase/nuclease of trypanosomatid protozoa. *Parasitology Today.* **5** (8): 257-260.

Hiroshi O, Tatsuro M, Setsuo T, Keiko T, Takekazu K and Masahiro K. 1983. Halophilic Nuclease of a Moderately Halophilic Bacillus sp**.** Production, Purification, and Characterization. *Appl Environ biol.* **45** (1): 24-30.

Howell HG, Brodfuehrer PR, Brundidge SP, Benigni DA and Sapino CJr. 1988. Antiviral Nucleosides A Stereospecific Total Synthesis of 2'-Fluoro-2'-deoxy-β-D-arabinofuranosyl Nucleosides; *J Org Chem.* **53**: 85-88.

Hui HZ, Jeong YC and Myung H. 2003. 8-Hydroxyguanine a product from damaged DNA can be used in the DNA salvage pathways; *Chinese Cheml Letters.* **14** 529 –532.

Jepsen PK, Riise E, Biedermann K, Kristensen PC and Emborg C. 1987. Two-level factorial screening for influence of temperature, pH, and aeration on production of Serratia marcescens nuclease. *Appl Environ biol.* **53** (10): 2593–2596.

Kamman JF and Tatini SR. 2006. Optimal conditions for assay of *Staphylococcal* nuclease; *Journal of Food Science*. **42** (2): 421 – 424.

Kobayashi H, Fumi K, Tadashi I, Takashi K and Norio I. 2000. Nuclease Lel 1 & 2 Study, *Biosci. Biotechnol. Biochem*. **64(**5):948-957.

Lachica RVF, Hoeprich PD and Franti CE. 1972. Convenient Assay for Staphylococcal Nuclease by the Metachromatic Well-Agar-Diffusion Technique*. Appl. Environ biology.* **24** (6): 920– 923.

Locke IC and Carpenter BG. 2004. Functional characteristics of the streptococcal deoxyribonuclease 'streptodornase', a protein with DNase activity present in the medicament Varidase; *Enzyme and bial technology.* **35** (1): 67-73.

Mishra NC. 2002. Nucleases-Molecular Biology and Applications Hoboken NJ USA John Wiley and Sons 189-190.

Motoaki A, Takakata H and Yasuwki T. 1970. A Deoxyribonuclease Which Requires Nucleoside Triphosphate from *coccus lysodeikticus* I Purification and characterization of Deoxyribonuclease activity; *J Biol Chem*. **245:** 767-774.

Peter HB and Tuan-Hua DH. 1986. Barley Aleurone Layers Secrete a Nuclease in Response to Gibberellic Acid Purification and Partial Characterization of the Associated Ribonuclease Deoxyribonuclease and 3'-Nucleotidase Activities. *Plant Physiol.* 82: 801–806.

Qin CF, Qin E, Yu M, Chen SP, Jiang T, Deng YQ, Duan HY and Zhao H. 2005. Therapeutic effects of dengue 2 virus capsid protein and staphylococcal nuclease fusion protein on dengue-infected cell cultures. *Archives of Virol.* **150**: 659-69.

Rangrajan S and Shankar V. 1999. Extracellular nuclease from Rhizopus stolonifer purification and characteristics of single strand preferential deoxyribose nuclease activity; *Biochim Biophys Acta*. **1473:** 293-304.

Robert VM and Alvin JC. 1976. Purification and Properties of Two Deoxyribonucleases of *Pseudomonas aeruginosa*; *J Bacteriol.* **127:** 794-802.

Schreier JB. 1969. Modification of deoxyribonuclease test medium for rapid identification of *Serratia marcescens*. *Am. J. Clin. Pathol*. **51**: 711 - 716.

Sluyser M. 2005. Apoptosis in normal development and cancer Taylor and Francis Group 47-48

Smith PB, Hancock GA and Rhoden DL. 1977. Improved medium for detecting Deoxyribonuclease producing bacteria. *Applied biology.* **18** (6): 991-993.

Sodek L, Wright STC and Wilson CM. 1970. Effect of EDTA on two RNases from wheat leaves. *Plant and Cell Physiology,* Vol. **11**(1): 167-171.

Sofia AK, Triantos AA, Papageorgiou GE, Kavoukopoulos EM, Vavatsi NA and Trakatellis AC. 1987. Single-strand-specific nuclease from rat liver endoplasmic reticulum: Characterization and mode of action. *International Journal of Biochemistry.* **19** (9): 857-864.

Tierney MB and Lamour KH. 2005. An Introduction to Reverse Genetic Tools for Investigating Gene Function. *The Plant Health Instructor* **1025:**01.

Verma KP, Saini A, Chopra AK, Chakraborti PK, Singh Y and Choudhary S. 2005. Nucleoside diphosphate kinase from *Mycobacterium tuberculosis* cleaves single stand DNA within the human C-myc promoter in an enzyme catalyzed reaction. *Nucleic acid Res.* **33** (8): 2707.

www.apsnet.org/Education/ReverseGenetics

Ying Guo-Qing, Lu-E Shi, Yi Yu, tang Zehn-Xing, Chen Jian Shu. 2006. Production, purification and characterization of nuclease p1 from *Penicillium citrinum*. *Process Biochemistry.* **41:**1276-1281.