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Effect of Incubation on DNase Production by a Moderate Thermophilic Bacterium Screened from Arid Land

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Wide varieties of organisms are used as a process organism to extract enzymes in industries. DNases has been used for commercial purposes apart from genetic engineering. For various applications the large scale production of DNases is important where a moderate thermophile is mostly preferred which decreases the cooling cost of the fermentation industries and also such enzymes can be stored at room temperature without denaturation. Keeping these objectives in view arid land in Patur region which belongs to Akola district was chosen as habitat for thermophiles. After screening large number of soil samples an efficient DNase producing moderate thermophilic bacterium was fully characterized. The morphological and biochemical properties were carried out and the phylogeny of this bacterium by 16S rRNA sequencing was studied. There are various parameters like composition of medium, degree of aeration, pH, temperature and incubation period which influence the DNase production. Every enzyme has its maximum time of production i.e. specific peak period, after which the production decreases. The production of the DNase enzyme started at 10 hrs and continued. The peak point of the enzyme was found at 29 hours and the enzyme production decreased thereafter.

Key words: Moderate thermophile, DNase production, Time point of production.

Deoxyribonucleases are a group of enzymes that are capable of hydrolyzing the phosphodiester linkages of nucleic acids. The hydrolysis of nucleic acids by the enzymes was observed for the first time by Araki in 1903[1]. The term nuclease was introduced by Iwanoff[2]. Kunitz classified nucleases into two major groups, ribonucleases and deoxyribonucleases, based on the specificity of their nucleolytic attack[3]. A new class of sugar-nonspecific nucleases was added to the list of nucleases which attacks on both DNA and RNA[4]. Nucleases are basically phosphodiesterase which act on the phosphodiester bonds of nucleic acids[4-5]. The nuclease enzyme is stable at 40° C for up to 3 months without loss of activity[6]. Many aspects of nucleases has been studied afterwards viz. staphylococcal nuclease differed from pancreatic deoxyribonuclease in that it split 5' bond leaving fragments with terminal phosphate groups at the 3' position and in that it requires calcium for activity[7]. Kamman and Tatini reported the optimum conditions for deoxyribonuclease obtained from one of the *Staphylococcus aureus* strains[8]. The temperature of 50°C and pH 10 were required for

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maximum nuclease activity. Enhancement in the nuclease activity is observed when calcium concentration of 0.005M and NaCl concentration of 0.17M was used. *Pedobacter ginsengisoli sp. nov*., a novel DNase producing bacterium was isolated from soil of a ginseng field in South Korea[9]. It is Gram-negative, strictly aerobic, rod-shaped, non-motile, non-spore-forming bacterial strain which was isolated in a soil sample from a ginseng field in Pocheon Province (South Korea) and was characterized by 16S rRNA gene sequence similarities. Deshmukh *et.al.,* studied extracellular deoxyribonuclease production by thermophile *Streptomyces thermonitrificans* which exhibited high extracellular DNase activity at pH 7.0 and 40°C [10]. Fox & Holimn studied the effect of anaerobiosis on *staphylococcal* nuclease production. Five strains of *staphylococcus aureus* for the production of nuclease under aerobic and anaerobic conditions were selected.

Hydrolysis of deoxyribonucleic acid and ribonucleic acid was detected by measuring the release of acid soluble nucleotides spectrophotometrically for determination of the effect of anaerobiosis on nuclease production[11]. Studies on evaluation of three methods using deoxyribonuclease production as a screening test for *Serratia marcescens* a characteristic feature which was found useful in distinguishing this organism from closely related members of the enterobacteriaceae[12]. With exploitation of living organisms for nuclease production synthesis of chemical nucleases was also attempted. One of such attempts was study of nucleolytic activities of two diverse classes of metalated catalysts, one nucleobase polymer and another bis-dipeptide conjugate, in the presence of exogenously added co-oxidant[13]. Although many factors affects DNase production like composition of medium, degree of aeration, pH, temperature but incubation period is most important because this enzyme is produced in later stages of the microbial growth curve. If this point is not studied properly the degradation of the enzyme will take place. Every enzyme has its maximum time of production i.e. specific peak period, after which the production decreases. This point of maximum DNase production was studied for a moderate thermophilic bacterial strain.

MATERIALS AND METHODS

Soil sample collection

Soil samples were collected from garden soil of Amravati University at specified depths up to 10cm using 2.54 cm. diameter soil corers. 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 [14].

Screening of DNase producing bacteria

The medium employed for DNase screening contained toludine blue and methyl green as indicator system where DNA hydrolysis was observed as decolorization of blue and green color[15-16].

Phylogenetic study of the culture

Genomic DNA was isolated from the pure culture pellet. The complete 1.4kb rDNA fragment was amplified using high –fidelity PCR polymerase. The PCR product was sequenced bi-directionally using the forward, reverse and internal primer. The sequence data was aligned and analyzed to identify the bacterium and its closest neighbors.

Enzyme production

Nutrient broth was prepared with 2% of DNA. Seven test tubes was taken and each test tube is filled with 5 ml of nutrient broth medium. Then the medium was inoculated with identified species. During incubation, each test tube one by one at the time interval of 5h kept in refrigerator. The extra cellular broth was collected by centrifugation (9000 x g. 30 min) and used as the source of enzyme. The effect of growth phase on the production of DNase in a broth culture was also studied[10].

Effect of incubation on DNase production:

After gelling, wells were cut for samples with high nuclease activity. Then enzyme containing broth was placed in the well cut in the agar. After incubation, zone of clearance around the wells was measured which corresponds to amount of DNase[17].

RESULTS AND DISCUSSION

Screening and morphological characterization of the bacterium

The screening was performed on DNase test agar with methyl green and toludine blue on

Table 1. Morphological and biochemical properties of the screened bacteria

Test	Result
Gram Staining	+
Motility	+
Endopsore	Central
Catalase	+
Urease	-
Oxidase	-
Indole	-
Methyl Red	-
Voges Proskauer	+
Citrate	+
Glucose	+
Lactose	-
Sucrose	+
D-Mannitol	+
Casein	+
Gelatin hydrolysis	+
Starch hydrolysis	+
Nitrate Reduction	+

the basis of decolorization of the media. The bacterium was found to be gram positive motile spore forming rod positive for methyl red test and Voges-Prauskeur test, acid was produced in glucose lactose and mannitol whereas gas was produced only in mannitol. Casein, gelatin and starch are hydrolyzed.

Table 2. Zone of DNA hydrolysis after particular time intervals

S. No	Time of Incubation	DNA hydrolysis
1	5 hr	0mm
2	10hr	13mm
3	15hr	14mm
4	20hr	16mm
5	25hr	16mm
6	28 hr	16mm
7	29hr	18mm
8	30hr	17mm
9	31hr	17mm
10	32hr	14mm

Phylogenetic study of the culture

When 16S rDNA sequencing was carried out the bacterium showed 99.5 % similarity to *Bacillus sp.* By113(B)Ydz-dh; EU070397. The bacterium also showed great similarity to *Bacillus aquimaris*.

Effect of incubation on DNase production

Effect of incubation on DNase production was studied it was found that the DNase production started from 10 hours, maximum was found at 29 hours and the production decreased thereafter.

It can be concluded that the bacterium characterized is a moderated thermophile able to

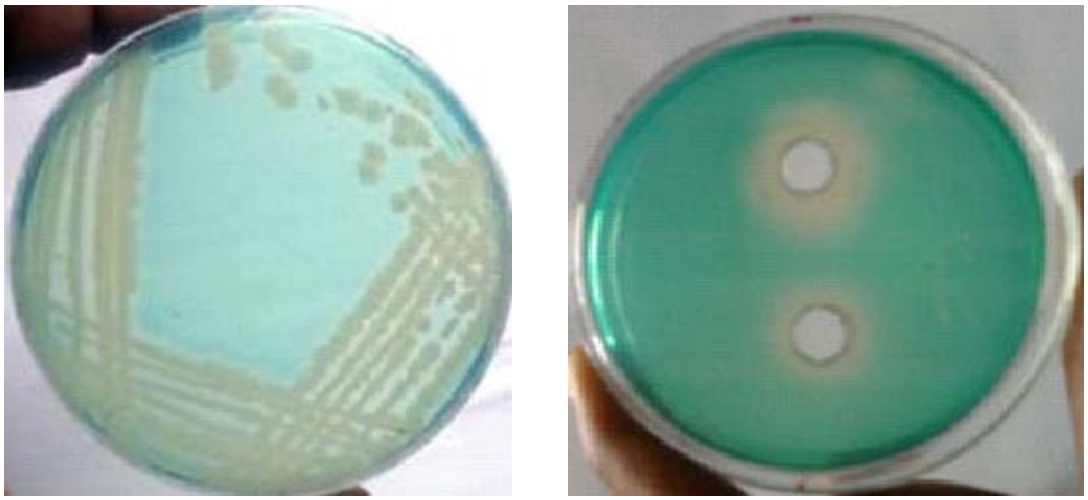

Fig. 1. Photographic plate showing screening of DNase producing bacteria and assay of DNase

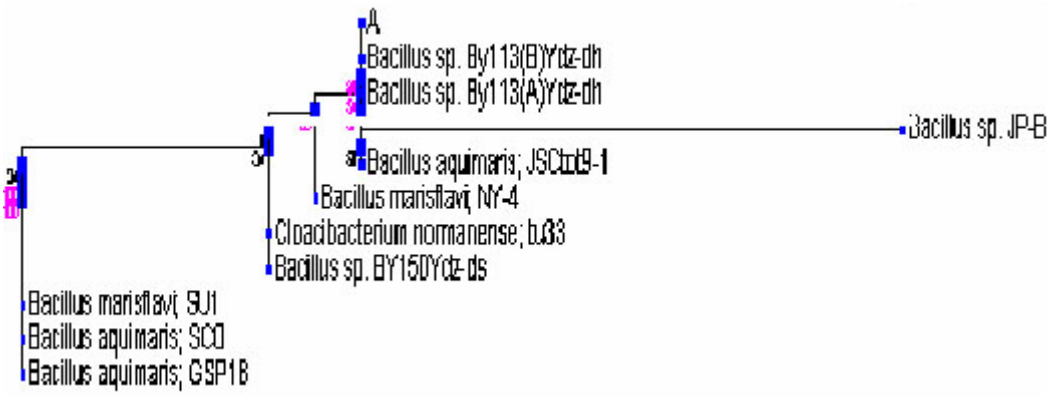

Fig. 2. The bacterium characterized is 'A' as shown in the phylogenetic tree.

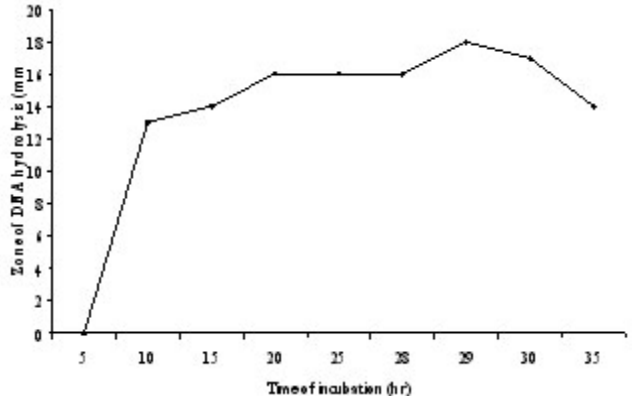

Fig. 3. Effect of incubation on DNase production

grow optimally at 45°C. The benefit of moderate thermophile is that strict higher temperatures are not required, at the same time can also grow at moderate temperatures. Because of the metabolic activities of the bacterium if temperature increases there is no need to decrease the temperature by coolants if large scale fermentations are carried out. The time point of DNase production studied in this regard is particularly important when the enzyme is to be purified so that the time of 29 hours is preferred for this step. The *Bacillus species* characterized can be used for DNase production anywhere.

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