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Article : Effect of aeration on deoxyribonuclease production by pigmented strain of Serratia marcescens

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ABATRACT:

Soil is the motherland of microorganisms; numbers of microorganism are found in soil which includes fungi; yeast, algae and bacteria. Each organism is able to produce an enzyme to survive in environment. Some organisms produce amylase, protease, lipase, nuclease etc. These organisms produce an enzyme broadly of two types extracellular and intracellular enzymes. The substrate for DNase producing bacteria in soil is decaying plant cells, animal cells and microbial cells. Deoxyribonucleases have great application in industrial as well as therapeutic fields. Our attempt was to isolate an efficient deoxyribonuclease producing bacteria from soil as soil is the richest source of microorganisms. Of the factors affecting the production of deoxyribonuclease, aeration is one of them. We have estimated the amount of oxygen required for maximum deoxyribonuclease production.

Keywords: Deoxyribonuclease production, *Serratia marcescens*, effect of aeration.

INTRODUCTION:

Deoxyribonucleases (DNases) are the enzymes which can break phosphodiester linkages of deoxyribonucleic acid (DNA). Although DNases are the part of every cell; there are very few microorganisms which produce DNases extracellularly. These extracellular DNases are relatively easy to purify. An extracellular nuclease was isolated from *Serratia marcescens* able to act both on DNA as well as RNA (Nestle and Roberts, 1969). The presence of disulfide bond was found essential for DNase activity in *Serratia marcescens*. The requirement for disulfide bond formation may play a role in preventing the lethal action of nuclease while in the bacterial cytoplasm (Ball *et. al.*, 1974).

DNase test is also used to differentiate *Serratia marcescens* from other bacteria belonging to Enterobacteriaceae. Many bacteria belonging to Enterobacteriaceae are found to be negative for DNase production (Black *et. al.*, 1971). The screening for DNase production is done on media containing DNA and methyl green is used as indicator (Smith *et. al.*, 1977) whereas assay can be performed on DNA agar with indicator only (Lachica *et. al.*, 1972).

Anaerobic bacteria have also been taken under study for deoxyribonuclease production either for characterization or production which includes *Fusobacterium species, Bacteroides fragilis, Clostribium species, Peptostreptococcus intermedium* and*P. anaerobius*. This can also show a potential pathogenicity of anaerobic bacteria along with some other enzymes (Porschen and Sonntag, 1974).

DNase has been studied from fungal plant pathogens viz. *Fusarium solani* produces a heat stable extracellular DNase which can break single or double stranded DNA (Gerhold *et. al.*, 1993). The activity was enhanced when fungus contracts pea endocarp tissue or when it is subjected to nutrient starvation in culture (Hadwiger *et. al.*, 1995.)

Serratia marcescens is a species of Gram-negative, rod-shaped bacterium in the family Enterobacteriaceae. *S. marcescens* is involved in nosocomial infections, particularly catheter-associated bacteremia, urinary tract infections and wound infections (Hejazi and Falkiner 1997).

A new (DNase) was purified from the cultured mycelia of *Cordyceps sinensis* which can degrade both double-stranded and single-stranded DNA but found to degrade dsDNA more. The optimum pH for this DNase is 5.5 and optimum temperature 55°C . The activity of DNase was inhibited by high concentration of the cation $MgCl_2$ above 150 mM, $MnCl_2$ above 200 mM, $ZnCl_2$ above 150 mM,$CaCl_2$ above 200 mM, NaCl above 300 mM, and KC1 above 300 mM. (Ye *et. al.*, 2004).

Extracellular DNase activity was studied in 73 strains of *Creptococcus neoformans* and 12 strains of *C. gattii DNase* production was higher in strains from clinical origin than among environmental strains. The enzyme had molecular weight of 31 kD. The extracellular DNase was considered to be involved in virulence factor in C. *neoformans* and *C. gattii* (Sanchez *et. al.*, 2010). Whatever be the source of microorganisms taken for DNase production, it is necessary to optimize the condition in order to enhance the DNase production and aeration is one such factor.

MATERIALS AND METHODS:

Preparation of Soil Suspension:

A known amount of soil (1g/1mm.) sample is suspended into the known volume of serial distilled water (9 ml). The total microbial suspension was adjusted 10 ml. Known volume of suspension (1ml) from 10 ml was transfer to additional test tube contained 9 ml to get serially diluted suspension of 10^{-1} to 10^{-10}. Aliquot of 0.1 ml suspension was poured on the surface of DNase test agar prepared for screening (Dubey, R.C. and Maheshwari, D.K.2008).

Screening of DNase Producing Bacteria: -

The soil samples collected by above mentioned method and regions were diluted by serial dilution technique and 0.1 ml was inoculated on nutrient agar. After colony count 10^{-7} dilution was selected. In most cases 10^{-7} dilution gave countable and well isolated colonies. These diluted soil samples were added on DNase test agar. Efficient DNase producers on the basis of DNA hydrolysis were chosen for further study. Screening was done on following media.

DNase test agar (Jeffries *et. al.*, 1957):-

This media employs the property of DNA to get precipitated on reaction with hydrochloric acid. The DNA salts readily dissolves in distilled water. The nucleic acid solutions are added to the liquefied agar just prior to autoclaving and the plates are poured as soon as the medium has cooled to 50°C. Inoculation of agar plates was done by adding 0.lml sample and was spreaded by sterile spreader. The inoculated media was incubated at 30°C in order to determine the maximal enzyme production. After incubation for 24 hrs, replicas of these plates were prepared. The enzymatic ctivity on the master plate was then assayed by flooding the plate with 1 N HC1. The acid reacts with the nucleate salts in the medium, yielding free nucleic acid, and consequently a cloudy appearance.

DNase test agar containing methyl green (Smith et al, 1977):-

Methyl green on reaction with DNA gives green color. In free form it is observed as colorless. This property is employed in designing DNase test agar containing methyl green. When DNA is hydrolyzed; the hydrolyzed area becomes colorless as methyl green gets free. Larger the colorless area around the colony; larger is the DNase production. On this media also 0.l ml from diluted sol sample was spreader

by sterile spreader. Media plates were incubated for 24 hrs at 30°C. Zone 0f DNA decolorization in fact DNA hydrolysis were noted.

Agar Well diffusion method for DNase Assay:-

Nutrient broth containing 0.2% DNA was taken in five conical flasks. One conical flask was kept as a control and other flask were placed on shaker at various rpm (at agitation phase), and one at stationary phase and were incubated at 37^0C for 24 hrs and later centrifuged at 13000 g for 30 min. Supernatant was taken as a source of DNase enzyme. From this tubes 80 µl was added in wells prepared on 8 mm DNase test agar and zone of DNA hydrolysis was observed. Larger the zone of DNA hydrolysis, larger is the amount of DNase production.

RESULTS AND DISCUSSIONS:

Fig 1:- DNA hydrolysis shown by *Serratia marcescens* strains on DNase test agar with and without methyl green.

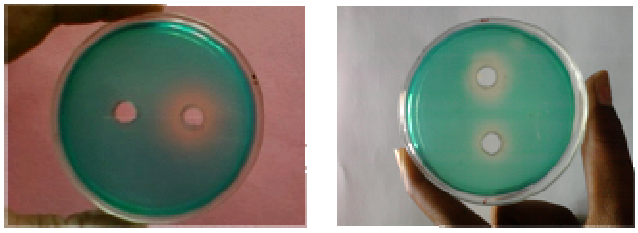


Fig 2:- Some of the plates showing DNase assay of the broth aerated at various RPM

Table1: Amount of dissolved oxygen at various RPM

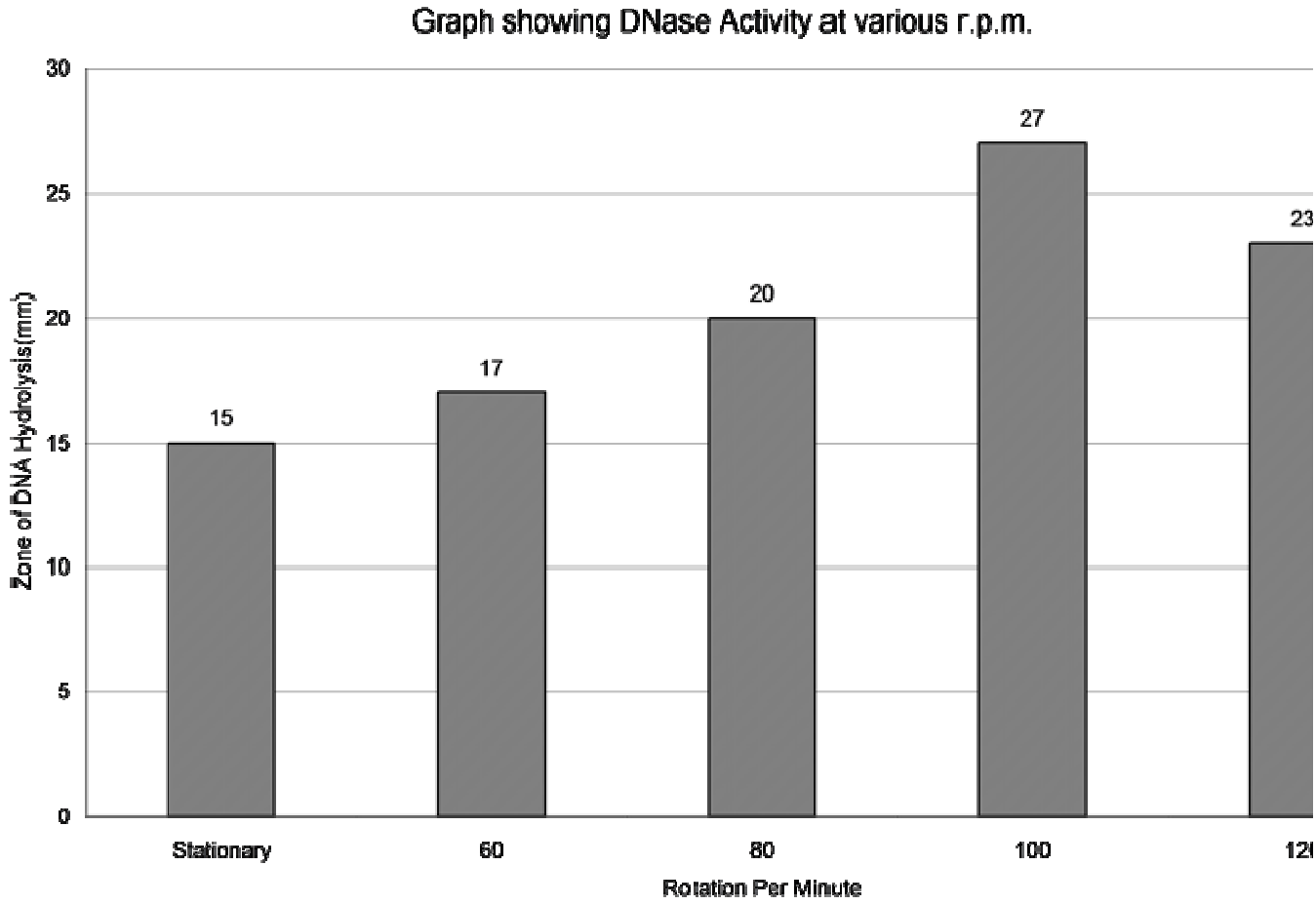
Sr. No	Aeration Condition	RPM	Dissolved oxygen (mg/l)
1	Rotary Phase	60	1.9 mg/l

2		80	2.7 mg/l
3		100	2.9 mg/l
4		120	3.1 mg/l
5	Stationary phase	-	1.2 mg/l

Table 2: Zone of DNA hydrolysis at various RPM

Sr. No	**Aeration Condition**	**RPM**	**Zone of DNA Hydrolysis (mm)**
1	Rotary Phase	60	17mm
2		80	20mm
3		100	27mm
4		120	23mm
5	Stationary phase	Stationary	15mm

Graph: Zone of DNA hydrolysis at various RPM.



The screening for DNase production was done on DNase test agar with methyl green. The production was observed on the basis of decolorization of methyl green as DNA after reaction with methyl green forms green color and when DNA is hydrolyzed the area becomes colorless. Later the DNase production was confirmed on DNase test agar by hydrochloric acid precipitation method. The hydrolyzed area is non- precipitated where as the unhydrolyzed DNA on reaction with HCl is precipitated. The culture on the basis of morphological and biochemical characteristics was found to be *Serratia marcescens*. Moreover, it is a pigmented strain of*Serratia marcescens* able to produce red colored pigment prodigiosin. The strain was grown in broth containing 0.2% DNA at stationary and aerated phase at various RPM for extracellular DNase production which was later centrifuged at 13,000g to separate the cells and the supernatant was taken as a source of extracellular DNase. In the stationary phase the amount of DNase was found to be very less with DNA hydrolysis zone of 15 mm followed by 17 mm hydrolysis zone

at 60 rpm and maximum at 100 rpm with 27 mm zone of DNA hydrolysis indicating that aeration at 100 rpm is necessary for maximum DNase production from *Serratia marcescens.*

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