

Prediction of Better Deoxyribonucleic Acid Hydrolase Producing Bacterium among Pigmented and Non-pigmented *Serratia marcescens* Strains from Soil

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ABSTRACT

Deoxyribonucleases (DNases) are the enzymes which break down the phosphodiester linkages of deoxyribonucleic acid (DNA) by addition of water and are categorized under hydrolases. They have large applications in almost all fields including therapeutic, industrial and genetic engineering fields. In present study *Serratia marcescens* strains were screened through a large number of soil samples. These strains were then studied for DNase production on DNase test agar and on the basis of zone of DNA hydrolysis around the colony; efficient two strains, a pigmented and non-pigmented strains were chosen further for comparative study. Attempt was to introduce a better DNase producer of the two strains to avail them for various applications. The morphological and biochemical properties were carried out and the phylogeny of this bacterium by 16S rRNA sequencing was studied in detail. The phylogenetic study and biochemical study confirmed that the strains belong to the genus *Serratia marcescens*. A quantitative estimation of the DNase was carried out which reveals that the non-pigmented strain is better DNase producing bacterium than the pigmented strain. The optimum conditions for both the strains were studied. Both strains produce the DNase optimally at 20°C and at pH 8. As both strains are isolated from soil; are non-pathogenic and can be handled with less care during the production. Further the pigmented *Serratia marcescens* produces prodigiosin a red colored pigment which may be responsible for lesser DNase productivity.

Key words: DNase production, Phylogenetic study, pigmented and non-pigmented *Serratia marcescens*.

INTRODUCTION

Nucleases are involved in 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). 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 Reactive Oxygen Species which is removed from DNA by endonucleases in DNA salvage pathways (Hui *et al.* 2003). Single strand specific endonuclease has been exploited in mutation studies (Tierney *et al.* 2005). In DNA foot printing 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 are also found useful against moloney murine leukemia virus. Derivatives of nucleotide products are prepared which mimics the viral activities (Howell *et al.*, 1988). Nucleases are also candidates for the enzymes producing a tasty

substance, 5'-GMP. (Kobayashi *et al.* 2000). Nucleases are studied from plants, insects and fungi.

In the present study *Serratia marcescens* strains were screened through a large number of soil samples. These strains were then studied for DNase production on DNase test agar and on the basis of zone of DNA hydrolysis around the colony; efficient two strains, a pigmented and non-pigmented strains were chosen further for comparative study.

MATERIAL AND METHODS

Soil Sample Collection (Banowetz *et al.*, 2006)

Soil samples were collected using a 2.54-cm-diameter soil corer. The corer was cleaned after each sampling 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 M. Banowetz *et al.*, 2006).

Screening of DNase Producing Bacteria (Schreier 1969)

Screening was done on DNase test agar with toluidine blue. Toluidine blue on reaction with DNA gives sky blue color. When DNA is hydrolyzed by bacterial action; toluidine becomes free and being in a free form a faint pink or colorless halos is observed around the colony.

Morphological and biochemical characterization of efficient DNase producers.

Routine microbiological methods were adopted for this purpose.

Phylogenetic study of some efficient cultures (Saitou and Nei (1987), Felsenstein (1985), Kimura (1980), Tamura *et al.*, (2007)

Identification by 16S rRNA sequencing is the most preferred these days. The service was provided by Chromous Biotech Pvt. Ltd. Bangalore.

Estimation of DNase (Lachica *et.al.*, 1972)

The characterized *Serratia marcescens* strains were grown in nutrient broth containing 0.2% DNA. The broth was centrifuged at 13,500 g to separate out the cells, so that supernatant is used as source of DNases as DNases are extracellular

enzymes. Wells were then cut on methyl green DNA agar by cork borer of 2 mm diameter. A measured quantity (3µl) of DNase was added by micropipette aseptically. These were then added in the wells prepared. Smaller DNase conc. formed smaller zones and higher DNase conc. formed larger zones of DNA hydrolysis as seen in photo plate NO. 3 and 4. A standard graph already prepared was referred for quantitative estimation of DNase in the broth.

RESULTS AND DISCUSSION

Screening of DNase producing bacteria



Plate 1: *Serratia marcescens* on DNase test DNase Test agar with toluidine blue

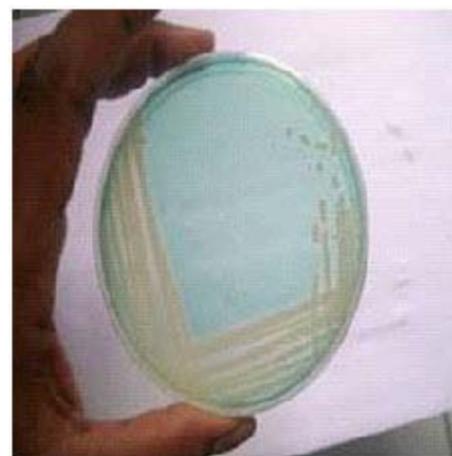
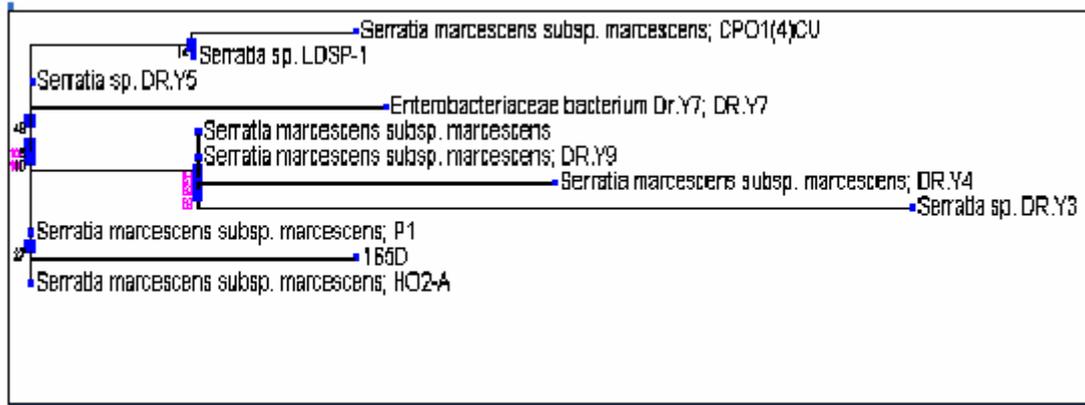
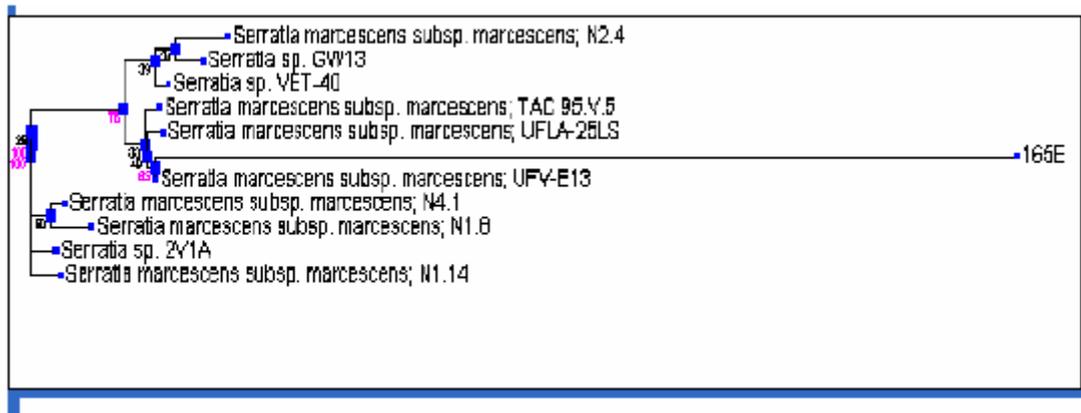


Plate 2: *Serratia marcescens* on DNase Test agar with toluidine blue

Phylogenetic study of the selected cultures



The pigmented strain in above phylogenetic tree is 165 D



The non-pigmented strain in above phylogenetic tree is 165 E

Estimation of DNase

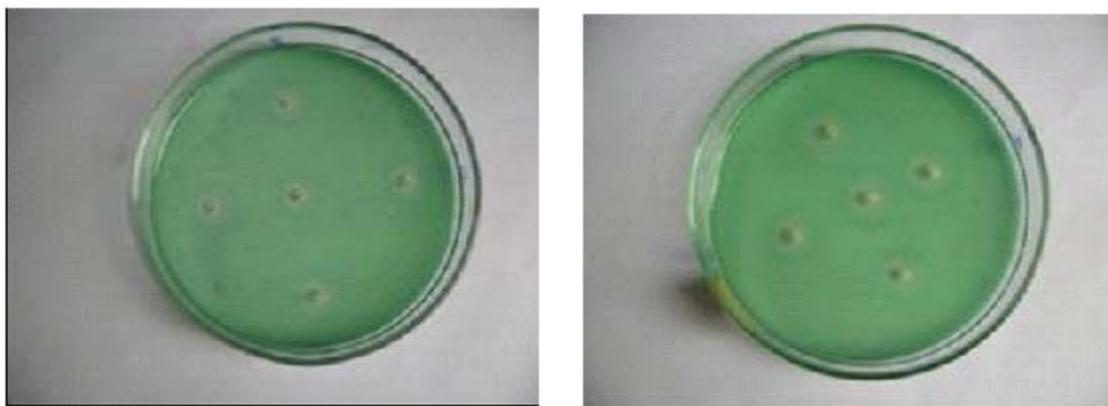


Plate 3 & 4: Metachromatic Agar Diffusion Assay of DNase

Serratia marcescens a pigmented strain produced maximum DNase i.e. 6.1µg/ml at pH 9 and 6.25 µg/ml at temperature of 20°C. Other non-pigmented strain produced maximum DNase at pH

9 i.e. 6.3 µg/ml and at temperature 30°C maximum DNase i.e. 6.35µg/ml was produced. This has shown that a non-pigmented strain of *Serratia marcescens* produces more DNase than pigmented strain.

Table 1: Morphological & Biochemical Characteristics of isolates from East region

Biochemical properties	<i>Serratia marcescens</i> (Pigmented)	<i>Serratia marcescens</i> (Non-pigmented)
Gram staining	Gram -	Gram-
Motility	+	+
Indole	-	-
MR test	+	+
Voges-Proskauer	-	-
Citrate	+	+
Hydrogen sulfide	-	-
Motility	+	+
Lysine decarboxylase	+	+
Ornithine decarboxylase	+	+
Glucose: acid	+	+
Glucose: gas	+	+
Lactose	-	-
Mannitol	+	+
Inositol	+	+
Arabinose	-	-
Raffinose	-	-
Rhamnose	-	-
Lipase (corn oil)	+	+
Cellobiose	+	+
Glycerol acid	+	+
Maltose	+	+
Glycerol gas	-	-
Gelatin	+	+
Pigment	+	-

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