PURIFICATION AND CHARACTERIZATION OF THERMOSTABLE DNases FROM BACTERIA IN HOT SPRINGS OF TRINCOMALEE, SRI LANKA

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Thermophiles are defined as organisms capable of living at high temperatures (hot springs and water heaters). Thermophilic bacteria are an interesting source of stable enzymes including thermo stable DNases. Nucleases played crucial role in development and use of recombinant DNA technology and the field of molecular biology. Currently there are approximately 3000 identified nucleases and recognized 235 different sequences. Most of them are found in bacteria and an estimated 25% of bacteria examined contain at least one restriction endo nuclease and therefore the probability of encountering new enzymes is relatively high. Objectives of this study were to isolate, purify and characterize DNA digesting enzymes from bacteria living in hot springs of Trincomalee, Sri Lanka.

Five different bacterial strains were identified based on the basic biochemical characteristics. All five strains belonged to the newly introduced genus *Geobacillus.* Based on the high growth rate, one strain was selected for studies on DNases. In this study, two DNA digesting enzymes, the high molecular weight DNase and the low molecular weight DNase, from the selected bacterial strain were purified to near homogeneity. Purification steps used were ion exchange chromatography using CM-Cellulose, Q Sepharose and DEAE cellulose, gel filtration chromatography using Sephacryl S-200, and affinity chromatography using Heparin-Sepharose. After

purification, enzymes were analyzed using SDS- PAGE to confirm their purity determine their molecular weights. Enzymatic properties of the purified DNases optimum pH, optimum temperature, thermal stability and metal ion requireme studied.

The purified high molecular weight DNase and the low molecular weight DNas estimated molecular weight of 66 kDa and 28 kDa according to SDS respectively. Both enzymes had an optimum pH of 7.80. Optimum temperature high molecular weight DNase and the low molecular weight DNase were 68 55^0C, respectively. Over 80 % residual activity was observed for the low m weight DNase incubated at 65 ^{0}C for 15 days while the high molecular weigh retained over 80 % activity at 60 ^{0}C for 15 days.

The activity of the high molecular weight DNase was enhanced in the presence ions while the activity of the low molecular weight DNase was enhanced in the p of Mg^{2+} ions. There was no absolute need of metal ions by both enzymes.

Further studies of both DNases using known DNA sequences are recommended to precisely identify their cleavage specificities. Moreover, studies on the am sequences and three dimensional structures of both enzymes are needed to rel unique thermal stability to structure.