Project Title: Assay conditions for the determination of NAD^+-Glyceraldehyde-3-phosphate dehydrogenase in thermophilic cyanobacteria *Synechococcus* sp.

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Abstract

Thermophilic cyanobacterium *Synechococcus* sp. represents one of the most interesting groups of micro-organisms in term of genetic mechemisms which enable them to grow and reproduce at high temperatures. The aim of this project is to find assay conditions for the determination of NAD^+-glyceraldehyde-3-phosphate dehydrogenase (NAD^+-GAPDH). The assay conditions will be used in the purification of the enzyme. *Synechococcus* sp. was grown in Medium D at 50°C in an incubator with 260 foot-candle light intensity for 12 hours per day. Flasks were shaken once a day for aeration. The result showed that cells reached mid log phase in 9 days. Mid-log phase cells were used for the assay of NAD^+-GAPDH. A suitable set of assay conditions consisted of the following assay medium : 2.4 ml assay buffer (30 mM pyrophosphate, 40 mM cysteine, pH 8.4; 0.1 ml aldolase (1 mg/ml); 0.2 ml 4 mM NAD^+; 0.1 ml 0.4 mM disodium arsenate; 0.1 ml enzyme solution. 0.1 ml 6 mM fructose-1,6-bisphosphate was added to the assay medium to start the reaction. Protein determination was by Bradford method. The amount of NADH produced at 28°C under the experimental conditions was found to be 3.2 n mole. mg. $protein^{-1}.min^{-1}$