Nitrogen assimilation system of red macroalgae: Enzymatic and physiological studies.

Alves-Lima, C.; Martins, A.P.; Marinho-Soriano, E.; Colepicolo, P.

1Dep. de Bioquímica, IQ, USP, SP, Brazil. 2Depto. de Oceanografia e Limnologia, CB, UFRN, RN, Brazil.

Red macroalgae (Rhodophyta) produce carrageenan, agar and high cost bioactive compounds of which production can be increased by a better understanding of their nitrogen metabolism. Nitrate reductase reduces nitrate into nitrite, which reduced to ammonium in order to be incorporated into aminoacids. Photosynthesis provides enough electrons and Carbon skeletons to complete this pathway. The goals of this study are: the optimization of the \textit{in vitro} cultivation of Brazilian algae \textit{Gracilaria birdiae}, establishment the nitrate reductase assay condition and to analyze the rates of photosynthesis. Enriched and sterilized seawater was used for cultivation at 25°C, 12:12 light/dark, 30 salinity, pH 8 and illumination of 65E.m\(^{-2}\).s\(^{-1}\). The relative daily growth rate in weight was used for the cultivation optimization. The enzymatic assay was prepared by adding nitrate and NADH to the enriched crude extract. After reaction stop, nitrite was detected through Griess reaction. Five parameters were used for the assay optimization: nitrate and NADH concentrations, time of reaction, temperature and pH. The photosynthesis was analyzed by the chlorophyll fluorescence which is directly proportional to the electrons transfer rate of PSII. The relative daily growth rate was 4.03%. The optimum enzymatic assay was: 3mmol.L\(^{-1}\) of NO\(_3\)\(^-\), 0.2mmol.L\(^{-1}\) of NADH and 10 minutes of incubation at pH 9 and 10°C. The maximum photosynthesis rate was 13.29E.m\(^{-2}\).s\(^{-1}\), the photosynthetic efficiency was 0.23, saturation irradiance was 65.66E.m\(^{-2}\).s\(^{-1}\) and the photoinhibition was 0.02. This study standardized the cultivation of \textit{G. birdiae}, the nitrate reductase enzymatic assay and the photosynthesis analysis, allowing more sophisticated studies on nitrate reductase enzymology and physiology.

Keywords: Nitrate \textit{reductase}, Gracilaria, \textit{photosynthesis}

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