Fluorescence Activated Cell Sorting (FACS) as a Tool for Rapid Establishment of Clonal Microalgal Cultures at the National Bank of Algae, Canary Islands, Spain

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## Abstract:

Isolation of microalgal strains to establish clonal cultures using traditional micromanipulation techniques, although effective, is normally time consuming, which limits the number of strains that can be isolated and catalogued in a culture collection. For biotechnological purposes, having the possibility of screening a high number of microalgal strains in short time increases the chance of finding suitable candidates for a given application e.g. high biomass production. The isolation step is clearly a bottleneck in this process of bioprospection. The recently created National Bank of Algae (Banco Nacional de Algas, BNA), at the Marine Biotechnology Center of the University of Las Palmas, based on the island of Gran Canaria, Canary Islands, Spain, is implementing fluorescence activate cell sorting (FACS) techniques to speed up the establishment of clonal cultures of eukaryotic and prokaryotic (cyanobacteria) microalgae. For that, BNA maintains a Beckman-Coulter Altra Epics flow cytometer equipped with both blue and red laser lines, a sense-in-crystal / sort-in-air droplet-deflection high speed sorting system, and standard chlorophyll, phycoerythrin, and allophycocyanin filter sets to discriminate and sort microalgal cells. The sorter has also a software-controlled robotic device (Cytoclone) that allows physical separation of single microalgal cells, colonies, or filaments, onto a variety of user-defined devices such as 96-well microtiter plates and agar plates, which facilitates the establishment of clonal cultures. Filamentous, mat-forming, and colonial cyanobacteria are pre-treated with ultrasonication prior to sorting, in order to generate filament or colony fragments that are small enough to be accommodated in the capillary lines and flowcell of the sorter, whereas single-celled microalgae are simply screened through a nylon mesh smaller than the orifice diameter of flow-cell tip. Ongoing investigations at BNA are focusing on recovery and viability of the cells after going through the sorting process. FACS, a technique developed for and traditionally used in biomedical research, is here presented as a powerful tool to help in the prospection of microalgal strains for biotechnological applications.

Key words: National Bank of Algae, BNA, microalgae, flow cytometry, Canary Islands