Simple and Efficient Method for Poly-3-hydroxybutyrate Quantification in Diazotrophic Bacteria within Five Minutes Using Flow Cytometry

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Abstract

Introduction. Polyhydroxybutyrate (PHB) is a reserve polyester stored by several bacteria as intracellular granules. PHB has thermoplastic properties similar to oil-based plastics, however is readily biodegradable in the environment. Because of its biotechnological potential, methods for PHB quantification are important to determine the better conditions of production in bacterial cultures. Often, PHB quantification is carried out by the laborious and time-consuming technique of whole-cell methanolysis followed by gas-chromatography (GC) analysis, which is impeditive to analyze a large number of samples at the same type. **Objectives.** Taken advantage of the PHB fluorescent staining by the dye Nile Red (NR), we established an efficient flow cytometry assay for PHB quantification in diazotrohic and plant-associated bacteria *Herbaspirillum seropedicae* and *Azospirillum brasilense*. **Materials and Methods**. Different membrane permeabilization solutions were assayed to produce a full uptake of NR. All staining conditions were optimized step-by-step, including ethanol percentage for cell permeabilization, time required for membrane permeabilization and NR exposure, dye concentration and stability. **Results and Discussion.** A high correlation coefficient (R^2 = 0.99) comparing both methods based on flow cytometry and GC was achieved. An extension of the applicability of the method based on flow cytometry was demonstrated quantifying PHB in epiphytic bacteria colonizing the surface of rice roots. This assay solves the three major limitations of GC-based analysis: (i) the use of hazardous solvents at high temperature, (ii) the long time required for sample preparation and analysis, and (iii) the high amount of sample. **Conclusions**. The time required to quantify stored PHB by flow cytometry does not exceed five minutes, and it is entirely compatible with fixed samples, without any loss in accuracy. In addition, the method can be applied with low amount of samples, allowing their use in epiphytic bacteria.

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