Abstract

In this paper we describe a cryopreservation protocol followed by the culture of Symbiodinium sp. isolated from the Caribbean gorgonian Pseudopterogorgia elisabethae as a potential renewable source of the dinoflagellate symbiont. Four different freezing protocols were designed: a controlled cooling device designed to cool at 1°C/min, a three-step protocol (−20°C for 2h, −70°C for 2h, liquid nitrogen-LN2), a two-step protocol (−70°C for 2h, LN2), and a one-step protocol (LN2). All cells were stored in LN2 after cryopreservation. The cryoprotective agents (CPA) used were ethanol (EtOH) and methanol (MeOH) at 10 and 20%, and seawater (FSW) was used as a control. Viability measurements using cell counts showed that all cryopreservation protocols were relatively successful, and no trends were observed regarding freezing protocol or CPA used. After 19weeks in culture the viability of samples which had high biomass was determined by the fluorescent assay CellTiter Blue™. The most viable cultures were those cryopreserved by a two-step protocol using 20% MeOH or 20% EtOH as a CPA. A genetic examination of the DNA of these samples using Symbiodinium-specific PCR primers confirmed that the composition of the culture had not changed. For the first time, we report that Symbiodinium sp. isolated from a gorgonian can be cryopreserved and subsequently cultured successfully.