Effects of temperature, photosynthetic photon flux density, photoperiod and O₂ and CO₂ concentrations on growth rates of the symbiotic dinoflagellate, *Amphidinium* sp.

Y. Kitaya · L. Xiao · A. Masuda · T. Ozawa · M. Tsuda · K. Omasa


Abstract Symbiotic dinoflagellates of the species *Amphidinium* are expected to be pharmaceutically useful microalgae because they produce antitumor macrolides. A microalgae production system with a large number of cells at a high density has been developed for the efficient production of macrolide compounds. In the present study, the effects of culture conditions on the cellular growth rate of dinoflagellates were investigated to determine the optimum culture conditions for obtaining high yields of microalgae. *Amphidinium* species was cultured under conditions with six temperature levels (21–35°C), six levels of photosynthetic photon flux density (15–70 μmol photons m⁻² s⁻¹), three levels of CO₂ concentration (0.02–0.1%), and three levels of O₂ concentration (0.2–21%). The number of cells cultured in a certain volume of solution was monitored microscopically and the cellular growth rate was expressed as the specific growth rate. The maximum specific growth rate was 0.022 h⁻¹ at a temperature of 26°C and O₂ concentration of 5%, and the specific growth rate was saturated at a CO₂ concentration of 0.05%, a photosynthetic photon flux density of 35 μmol photons m⁻² s⁻¹ and a photoperiod of 12 h day⁻¹ upon increasing each environmental parameter. The results demonstrate that *Amphidinium* species can multiply efficiently under conditions of relatively low light intensity and low O₂ concentration.

Keywords Algae · *Amphidinium* sp. · Photosynthetic photon flux density · Specific growth rate · Symbiotic dinoflagellate · Temperature

Introduction

The amphidinolides are a series of unique cytotoxic macrolides isolated from the dinoflagellate *Amphidinium* sp. that has been separated from marine acoel flatworms of the genus *Amphiscolops* (Kobayashi and Tsuda 2004; Kobayashi and Kubota 2007). Up to now, 39 amphidinolides have been reported (Oguchi et al. 2007). Amphidinolides H (Kobayashi et al. 1991, 2000) and B (Ishibashi et al. 1987; Kobayashi et al. 1989; Bauer et al. 1994), which were initially isolated from species of the marine dinoflagellate *Amphidinium* (strains Y-25 and Y-5, respectively), are 26-membered macrolides possessing unique structures such as an allyl epoxide and an S-cis-diene, and exhibit potent cytotoxicity (IC₅₀ 0.0045–0.00014 mg mL⁻¹) against cultured tumor cells in vitro and antitumor activities in vivo. More recently, a new series of potently cytotoxic *Amphidinium* macrolides, designated iriomoteolides, have been isolated from the benthic *Amphidinium* strain HYA024 (Tsuda et al. 2007a, b). These macrolides are, therefore,
expected to be good candidates for antitumor drugs with a novel mechanism of action because their chemical structure is unlike that of conventional anticancer materials. Nevertheless, development of these compounds as anticancer drugs has not been smooth due to limited sample amounts.

Development of a production system for *Amphidinium* cells separated from *Amphiscolops* is desirable in order to produce macrolide compounds efficiently. However, the construction of a mass culture system for *Amphidinium* production has not yet been established because environmental control of the mass culture of *Amphidinium* cells at a high density is difficult using conventional culture methods. Microalgal cell growth rates are affected by environmental parameters such as temperature, light intensity and period, and gas composition in the culture system, as well as the combination of all these factors. Few papers have focused on environmental control of cell cultures of symbiotic *Amphidinium*. In order to establish a symbiotic *Amphidinium* production system with a large number of cells and a rapid turnover rate, the appropriate combination of environmental parameters needs to be determined.

This research was initiated to optimize culture condition for symbiotic *Amphidinium* production. The present study is a starting point for the optimization of the culture. Effects of variable culture conditions on the cell growth rate of dinoflagellates were investigated to determine the potentially optimum culture conditions for obtaining high yields of this microalga. Effects of temperature, photosynthetic photon flux density, photoperiod and CO₂ and O₂ concentrations on growth rates of dinoflagellate cells were assessed using a hanging-drop culture system.

### Materials and methods

The dinoflagellate *Amphidinium* sp. (strain HYA002) was separated from the internal cells of the marine acoel...