INTRODUCTION

Unicellular microalgae of the genus Porphyridium are primitive representatives of Bangiophyceae (Rhodophyta). The species Porphyridium purpureum (Bory de Saint-Vincent) K.M. Drew et R. Ross, 1965 [synonyms are Porphyridium cruentum (Gray) Nägeli, 1849 and P. marinum Kylin, 1937] is interesting as a producer of polyunsaturated fatty acids (arachidonic and eicosapentaenoic, which are precursors of prostaglandins and gel-forming polysaccharide) that rarely occur in biological objects [15, 19–21, 39, 40, 45]. The high diversity of fatty acids and phycobilins and the relatively simple cultivation method make P. purpureum an interesting subject for the study of the roles of pigments in photosynthesis and the role of lipids in the formation of the photosynthetic system [6, 8]. In this regard, much attention is paid in the scientific literature to the biotechnology and biochemical aspects of P. purpureum [6, 21, 28, 38]. The influence of cultivation conditions on the growth of P. purpureum has been analyzed and reliable cryopreservation techniques have been suggested in many studies [17, 29, 41]. Cryopreservation is very important for long-term storage of algae cultures, which helps to avoid changes that result from repeated subcultivations, probable contamination, or genetic drift [22–24]. Some attempts to freeze algae of the genus Porphyridium have been made [35, 41, 44], but the obtained information on the influence of different cryopreservation modes and cryoprotectants on the growth of algae in laboratory cultures is rather contradictory.

Therefore, the present study focuses on the growth peculiarities of the red alga P. purpureum in a laboratory culture that was isolated from waters of the Sea of Japan, in different culture media and at various water salinities. We also analyzed the ability of its cells to recover after cryopreservation.

MATERIALS AND METHODS

A culture of P. purpureum was isolated from a seawater sample collected from the surface horizon (0–0.5 m) of Amursky Bay in October 2012. The salinity of the seawater was 32.6 vol %; the water temperature was 10–11°C. We used the previously published methodical studies to obtain a clone culture of the alga [11, 12, 31]. First, we obtained a mixed culture before isolating the rare species, then 50 mL of seawater sample were added to 50 mL of f medium [31], mixed thoroughly, distributed over sterile 6-cm Petri dishes, and placed in a chamber for cultivation of the algae. Cells of P. purpureum were found in one dish with mixed culture after 15 days. A single cell was isolated with a capillary pipette, washed in sterile seawater, and placed into f medium. The clone culture of P. purpureum was maintained in a viable state by regular (each 10–14 days) reseeding to fresh media under...
the standard conditions: at the temperature of 20 ± 2°C, illuminance of 3500 lx on the flask surface, and a 12 h light : 12 h dark illumination regime. This regime was constant to obtain a synchronous culture [25].

The growth dynamics of *P. purpureum* were studied not only in f medium, but also in Goldberg’s medium modified by Yu.G. Kabanova [9]. To compare the cell density dynamics during cultivation in both media, 100 mL of each media were poured into two Erlenmeier’s flasks and then the algal cell suspensions from the corresponding media were added in each flask in such a way as to have the initial cell density of 4 × 10⁴ cells/mL. The experiment lasted for 14 days. Samples for counting cells per unit of volume were collected from each flask after thorough stirring each 2 days at the same time. The cells were counted in a Goryaev’s chamber. The material was studied and photographed under an Olympus BX 41 light microscope (Tokyo, Japan).

The influence of salinity decrease on *P. purpureum* cell density was analyzed by cultivating it at salinity levels ranging from 4 to 32 vol % with the intervals of 4 vol % and 2 vol %. The growth curves reliably show its dependence on salinity when this interval is used [7]. The growth at 32 vol % salinity, which is close to the seawater salinity in natural habitats of the species, was used as the control. The seawater was diluted with distilled water and sterilized to prepare media with a salinity lower than the control [27]. The salinity was measured with a GM-65M salinity meter. Then solutions with different salinities were enriched with the nutrient components of f medium [31]. The primary culture for inoculation was cultivated under the standard conditions described above. The inoculum was used at the exponential growth phase. The primary culture was centrifuged at 900 g for 5 min (an OPN-8 centrifuge, Dastan, Kyrgyzstan) to reach a high density of inoculation cells. The initial cell density in the experiments was 4 × 10⁴ cells/mL. Sample collection and cell counting were conducted as described above. The growth rate and generation time were determined through corresponding equations [63]. The average size of 30 cells at each salinity was calculated.

The influence of freezing on the viability of *P. purpureum* cells was studied by using the algal culture at the stationary phase. The cell suspension was diluted with f medium to reach a concentration of 4–5 × 10⁶ cells/mL before freezing. Microalgae were frozen to the temperature of −196°C using penetrating (dimethyl sulfoxide, ethylene glycol) and non-penetrating cryoprotectants (trehalose) at the final concentration of 2.5% (vol/vol) and 20 mg/mL, respectively. The cryoprotective solutions were prepared using sterile seawater. The cell suspension (0.6 mL) was transferred into 2-mL cryovial tubes, and 1.2 mL of cryoprotective solution was added during 5–7 minutes. One-, two-, and three-step freezing regimes were tested. For the one-step freezing, the cryotubes were immersed into liquid nitrogen (LN) and the freezing rate was 90–100°C/min. The two-step freezing was performed, first, in a low-temperature Proline RP 845 thermostatic circulator (LAUDA, Germany) to −25°C at a rate of 1–1.3°C/min for 30 min and then the cryotubes were immersed in LN. To perform the three-step freezing, cryotubes were cooled to −25°C at a rate of 1–1.3°C/min for 30 min in a low-temperature thermostatic circulator. They were then transferred to a “raft” floating on the surface of the LN, where they were cooled to −75°C at a rate of 1.8–2°C/min for 20 min; after this the cryotubes were frozen in LN for storage. The permanent temperature control was conducted with a TC-08 thermocouple logger (Omega Engineering, United States) at a frequency of 10 measures/s.

After storage in LN for 1–30 days, the cryotubes were immersed in a circulating water bath at 30°C. Immediately after thawing, the contents of the cryotubes were transferred into sterile centrifuge tubes, gradually diluted up to ten times in sterile seawater, and precipitated in an Allegro X-22R centrifuge (Beckman-Coulter, United States) at 900 g for 5 min. The washing procedure was repeated, then 1.8 mL of the nutrient medium was added to the pellet and cells were cultivated under standard conditions. The cell concentration was determined using a light microscope every 3 days for 2 weeks, as described above. Unfrozen algal cells at the stationary growth phase were used as the control; they were diluted with the nutrient medium to the concentration of 1 × 10⁶ cells/mL and cultivated under the same conditions as cells after cryopreservation. Each experiment was performed in triplicate.

**RESULTS AND DISCUSSION**

Until now *Porphyridium purpureum* has been studied by using strains isolated from coastal waters of Europe, United States, and Asian countries and stored in different collections of microalgae cultures [5, 34, 43]. We isolated the first strain of unialgal culture of *P. purpureum* from the northwestern Sea of Japan, i.e., from the northern border of the species range in Asian waters.

When studying *P. purpureum* cultures under a light microscope, we observed single spherical or slightly ellipsoid cells (Fig. 1) and small aggregates of 5–8 cells. Each cell had one star-shaped chloroplast with a pyrenoid located in the center. The diameter of the cells varied from 6 to 12 µm; the average cell size was 6.3 ± 1.4 µm.

The growth curves of the accumulative culture were characterized by phase changes as in all cell cultures [11, 13, 26, 42]. The cell-density dynamics of *P. purpureum* almost did not differ between cultures on different media (Fig. 2). The average growth rates at the exponential phase (4th day) in f medium and Goldberg’s medium were 0.52 and 0.50 divisions per day.