

The Contemporary Methods of Maintenance of Algal and Blue-green Algal Cultures

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The collections of algal and blue-green algal cultures have the same functions and objectives as the collections of the other microorganisms — i. e. to secure long-term strain maintenance without changes of their identity. Compared with the collections of heterotrophic microorganisms, in the algal collections there is necessary to respect some peculiarities arising namely from the autotrophic nature of algae and blue-green algae, i. e. the fact that the light source is essential for their growth. Moreover, the algae are mostly very sensitive to changes of life conditions, and this is why the maintenance of their strains (providing the stability of all diagnostic characters and properties) is sometimes more complicated than in the case of heterotrophic organisms. The up-to-now methodical approaches are similar, yet the applicability of new, progressive methods for the algae and blue-green algae is considerably selective and limited.

The most common method used for long-term maintenance of algal and blue-green algal strains is the classical one („subculturing“) consisting in maintaining growth active strains on solid (agar), liquid or biphasic media by repeated inoculation and cultivation under suitable light and temperature conditions (Pringsheim 1946, 1950, 1951, Provasoli et Pintner 1960, Stein 1973, etc.).

The drawback of this method consists in the first place in laborious re-inoculation and medium preparation, especially when subculturing a larger number of strains of various systematic groups. As for the nutritional and other requirements (light, temperature, pH) not only the individual systematic groups of algae, but so sometimes even the individual strains of the same species mutually differ — see also Leeson et al. (1984, p. 132): „Individual strains may show different requirements vary not only between groups but also from strain to strain, and extensive trials are required to find the best medium for individual algae.“ Due to highly specific or unknown requirements it has not so far been possible to obtain the cultures of certain species of algae or blue-green algae

(e. g. many diatoms, epiphytic algae, some blue-green algae forming „water-blooms“, etc.).

As the nutritional requirements and thus also the limiting concentration of biogenic elements of most algae and blue-green algae are not exactly known the used media are mostly complex. Many of them contain organic components (soil, peat or yeast extract, vitamins, urea, etc.); this enhances the possibility of contamination by heterotrophic microorganisms (bacteria, moulds, etc.). Due to primary production of organic matter the condition for the multiplication of heterotrophic contaminants are rendered even more advantageous, as they can utilize the products of photosynthesis of algae or blue-green algae.

The advantageous use of selective media for the elimination of the contaminants is minimal (as compared e. g. with the utilization in bacteriology). When antibiotics inhibiting growth and multiplication of heterotrophic microorganisms are applied (Spencer 1952, Droop 1967, Bednářová et al. 1976) genetical alternations in the algal cells cannot be eliminated. Moreover, the contaminants are more resistant against these preparations than the algae.

For the above reasons obtaining and long-term maintenance of axenic cultures of algae and blue-green algae strictly require conditions of aseptic work and the pertinent laboratory equipment (Brown et Bischoff 1962, Soli 1964, Starr 1973, etc.).

As the culture media need not always be optimal the strains cultivated for a longer time in unsuitable life conditions can change their characteristics in consequence of the selection (see Leeson et al. 1984). This constitutes the additional negative feature of the traditional cultivation method.

Despite all the above disadvantages the classical method of maintaining algal and blue-green algal strains is still the most used because of its universal applicability almost for all systematic groups. The undisputed advantage of the method can be seen in the readiness of growth active cultures.

Certain progressive methods nowadays currently employed in the collections of heterotrophic microorganisms try to remove the imperfections of the classical method of maintenance of algal cultures. These methods are based on some of the following principles: decreasing of growth activity of the cells (method of immobilization), vacuum drying of the cells after preceding freezing (lyophilization, freeze-drying) or freezing with subsequent cell or culture maintenance in very low temperatures (freezing).

Viability of cultures of microorganisms preserved by one of the above-mentioned methods depends on many factors — both „intrinsic“ (cellular) and „extrinsic“ (physical). These factors and their mutual interactions play specific role in the individual organisms.

In algae and blue-green algae (see Leeson et al. 1984) the results of the

lyophilization method and freezing depend mostly on the choice of cellular material (e. g. resting stages are more resistant than the vegetative forms), capability of cold acclimatization (i. e. specific resistance against freezing), age of culture (cells from exponential growth phase are more sensitive than those from the stationary phase), culture conditions before and after freezing (i. e. growth medium, addition of metabolic inhibitors, temperature, etc.), concentration and type of the cryoprotective, rates of cooling and warming, final storage temperature, etc.

From the above-mentioned reasons many attempts at suitably applying progressive methods for algal and blue-green algal cultures met with numerous difficulties.

Experience with the use of algal cell immobilization based on the principle of decreasing of growth activity of the cells leading to blockade of the reproduction process are so far only sporadic. Recent experiments have shown that in the cells of chlorococcal algae the morphological changes visible in LM as well as EM take place. In some algae there appear even considerable deformations of the external cell morphology (Lukavský et al. 1986).

Nevertheless, the viability of the immobilized algae or blue-green algae remains good. Of 30 various strains immobilized 32 month into 2% agar the viability was again restored in 90% after transferring them into a fresh culture medium (Lukavský, in press).

Simple performance and minimal financial costs are considered the main advantages of agar immobilization.

The lyophilization method based on the principle of vacuum drying of the cells after preceding freezing was tested in algae and blue-green algae in the fifties (Daily et Mc Guire 1954, Watanabe 1959). Further experiments were conducted in the first place by American algologists (Meryman et al. 1960, Holm-Hansen 1963b, 1964, 1967, 1973, Tsuru 1973, Alexander et al. 1980, etc.).

The most suitable cryoprotectives in blue-green algae and algae are considered 10% skim milk, sometimes with the addition of 1% monosodium glutamate, 12% saccharose, horse or lamb serum. The optimum drying time varies between several hours and several (approx. 5) days. For storing of the lyophilized strains temperature lower than +5 °C, for some algae as low as -60 °C (Alexander et al. 1980) is recommended. Other authors achieved satisfactorily results at RT and recommend to store only some algae at lower temperature (e. g. Holm-Hansen 1973).

The main objection against the use of the lyophilization method in algal collection is the very low viability of the thus preserved strains. Already after 24 hrs. it is usually impossible to revitalize more than a tenth of the percentage

of the cells, in the best cases several per cent. After a longer time the viability of most cultures is very low (lower than 1%), the results of experiments performed in blue-green algae cultures being always better than those in algal cultures (Leeson et al. 1984).

Thus, as compared with many heterotrophic microorganisms (especially the sporulating ones) the above approach to long-term strain maintenance did not prove suitable, yet it can be employed for maintaining of the biomass of algal material for chemical or biochemical analyses.

Much more favourable results in maintaining of algal and blue-green algal cultures were obtained when the method of freezing with subsequent storage of the frozen strains in liquid nitrogen or its vapors was employed. This method was practically tested in the fifties and sixties, yet mainly in the seventies and eighties (Leeson et al. 1984).

The most suitable cryoprotectives in blue-green algae and algae are considered glycerol (5% or 10% v/v), MeSO = dimethylsulfoxid and methanol (2.5 M). Freezing of the algal strains is performed either in freezing boxes or automatic freezing units which proved more suitable in the first place because the freezing rate than the optimum one is employed.

For a recovery of the viable algal material the average warming rate should be ± 90 °C/min and defreezing must be completed within 40—120 sec, otherwise the cells are damaged.

If cryoprotectives must be used in the application of the method, it is essential in revitalizing the culture to remove it from the medium by repeated washing, in order to prevent inhibition of further culture growth.

Although the percentage of viable cells depends on numerous factors (Holm-Hansen 1973, Tsuru 1973, Schwarze 1975, Morris 1976a, 1976b, 1978, Mc Grath et Daggett 1977, Alexander et al. 1980, Leeson et al. 1984, etc.) the results achieved are much more better than those when lyophilization was used. In optimum performance the average viability after 1 day to 3 months was higher than 50%, in some cases even 100% (Leeson et al. 1984).

Good results achieved in several years' study and testing by English and American pioneers of this method (Morris and Holm-Hansen) led to its practical implementation for long-term maintenance of many algal strains (*Chlorococcales* and *Euglenophyceae*) deposited in CCAP (Culture Centre of Algae and Protozoa, Cambridge). Leeson et al. (1984, p. 156) report: „At CCAP, a recovery of 60 % is the minimum accepted for long-term maintenance . . . , over 400 strains of algae are now successfully stored under liquid nitrogen.“

The method of culture freezing with subsequent storage of the material in liquid nitrogen is doubtlessly the most perspective method the use of which in the collections of algal and blue-green algal cultures will be ever increasing,

as various difficulties connected with its application will be overcome. One can nevertheless assume that the method will hardly be universally applicable for all strains of algae and blue-green algae, especially for such with poor adaptability to low temperatures. Yet, the above modern approach represents a considerable progress and facilitation of the work for all those concerned with maintaining of the unique strains of microorganisms thus contributing to the protection of the gene pool.

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