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Effect of temperature on growth and bioactivity of two *Nostoc* strains in mass culture

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Chemical and pharmacological studies of cyanobacterial bioactive substances are often limited by the availability of the metabolite. For complex bioactive molecules that defy economic chemical synthesis, cultivation of the producer strain is generally the only solution to provide enough material for screening (clinical and pre-clinical tests, field trials for agriculture). In addition to the usual problems encountered in mass cultivation of cyanobacteria, cultivation of bioactive strains implies several difficulties, among which loss, alteration or decrease of bioactivity, toxicity against operators, treatment of the exhaust medium and, above all, defining conditions for maximum metabolite production.

Only few studies on the mass production of bioactive cyanobacteria have been published. In most cases scale-up has been achieved by a number of small (15-30 L) square pans or carboys (Patterson *et al.*, 1991, J. Phycol. 27: 530-536; Rossi *et al.*, 1997, J. Appl. Phycol. 9: 195-204). A 300 L tank has been used to study cytotoxin production by a marine *Lyngbya* strain (Armstrong *et al.*, 1991, J. Appl. Phycol. 3: 277 – 282), and a 50 L tubular photobioreactor for cultivation of a toxic strain of *Nodularia harveyana* (Pushparaj *et al.*, 1994, J. Appl. Phycol. 6: 533 - 537). A cost-effective 800 L HDPE cylindrical tank was built and tested for production of cytotoxins from two cyanobacterial strains (Bolis *et al.*, 1999, XVI Int. Bot. Congress). Little information is available on the factors influencing bioactivity of cultured organisms in mass culture.

Two *Nostoc* strains (ATCC 53789 and our isolate Mz1) were cultivated in six 120 L annular reactors. The 4 cm wide annular photostage of this reactor was made by joining at the base two Plexiglas cylinders of 40 and 50 cm in diameter placed vertically one inside the other. Continuous artificial illumination (175 µmol photons m^{-2} s^{-1}) was provided on the surface of the inner cylinder by six 58W fluorescent lamps. The external cylinder was wrapped with black plastic to exclude natural light from the culture. Mixing, oxygen degassing and pH regulation were achieved by bubbling a CO_2/air mixture (2/98; v/v).

Growth and productivity of the two strains were evaluated in batch and semi-batch cultures at three different temperatures and in two different culture media (BG11 and $BG11_0$). Bioactivity of the biomass and of the culture medium along the growth curve was evaluated using 96-well microtiter plates and *Penicillium expansum* as the target organism.

Temperature and medium composition strongly influenced productivity and bioactivity of both strains. Productivity during the linear phase varied between 0.2 and 0.4 g (d.wt) L^{-1} d^{-1}. Typically, bioactivity accumulated in the early linear phase of growth and decreased in old cultures. The culture media showed low or no activity. Differences between the strains are discussed.

Mass production of bioactive biomass of phototrophs is generally much more expensive than that of chemoheterotrophs. The study of the factors that maximize growth and productivity of secondary metabolites, together with the development of cost-effective reactors, is crucial for the commercial exploitation of bioactive substances produced by cyanobacteria.