N-CARBOXYMETHYLCHITOSAN: ALGISTATIC AND ALGICIDAL PROPERTIES

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SUMMARY: A water soluble agent made from waste crustacean shells that controls the growth of algal cells was identified. Growth of an Anabaena sp. was inhibited in the presence of varied concentrations of a chitosan-derivative, N-carboxymethylchitosan (NCMC). Application of a dilute water solutions of NCMC prevented algae from multiplication and enhanced aggregation of cells and assured clearing of algal suspension by filtration.

INTRODUCTION

Anabaena spp. (Cyanophyceae) are among the most common algae in fresh water environments such as swimming pools, aqueducts, sewages and natural water sources. Presence of algae in aqueous environments modify the environmental conditions by their growth and metabolite production and can produce undesirable consequences.

Chitosan is a cationic carbohydrate polymer of B-1,4 glucosamine residues that is chemically derived by acetylation of natural-occurring chitin; the parent material is obtained primarily from crustacean shells (Muzzarelli et al., 1982, Muzzarelli, 1988). The chitosan derivative, NCMC, is more water soluble than native chitosan. Although previous studies have demonstrated the antimicrobial effect of chitosan (Allan and Hadwiger, 1979, Hadwiger et al., 1984, Muzzarelli, 1988), there is no report in the literature on chitosan as an algistatic or algicidal agent. The main objective of the current study was to acquire basic information on the effects of NCMC on algal development.

MATERIALS AND METHODS

Algal culture conditions: Pure culture of an Anabaena sp. was obtained from the Food Flavor Quality Laboratory, USDA-ARS-SRRC, New Orleans, LA, and grown on the Modified Allen’s medium for blue-green algae (Vonshak, 1986). Stock cultures were maintained at 24 C, continuous light (9.3 lx).

Algal culture treatments: NCMC was provided by Dr. J. Vercellotti. Previous carboxymethylation of chitosan had been achieved by reacting the free amino groups of chitosan with glyoxylic acid to produce a soluble, gel-forming imine and reduction with cyanoborohydride (Muzzarelli, 1988). The chitosan produced for the
The current study utilizes sodium borohydride at pH 5 as a reducing agent. For each experiment, fresh algal inocula were used; cell densities (10^6 cell per ml) were determined microscopically with a haemocytometer. Six replicates of 50 ml axenic algal culture were amended with NCMC for each treatment. Varied concentrations (0, 12, 24, and 39 nM) of NCMC were used in each culture (total volume = 51 ml). NCMC was added to the medium simultaneously with algal cells (day 1) in one set of tests. In another group of experiments, the effect of NCMC on mature cells was determined by adding the compound to a 9-day old algal culture; the culture was incubated and sampled for pH, biomass, and chlorophyll determination after 6 days of chitosan treatment (15 days total incubation). The pH of the culture medium and controls was initially adjusted to 8.1 after addition of NCMC.

Incubation and sampling: Algae were incubated as shake cultures (100 rev/min-orbit Instrument) at 22 C under continuous illumination at 2,500 lx (Rheem Environmental Chamber). Determination of biomass (dry weight), pH, and chlorophyll production were carried out along with microscopic observations (electronic and light compound microscope) at day 1, 2, 6, and 9 of incubation. For the 9 day cultures, pH, biomass, and chlorophyll determination were carried out after 6 days (15 days total incubation).

Biomass determination: Cells were separated from the medium by membrane filtration (glass fiber filter, 0.45 μ) and washed with distilled, sterile water to remove salts and residues. Cells retained on weighed filter membrane were vacuum-dried overnight, cooled in a desiccator to room temperature and weighed immediately. Chlorophyll determination: A modification of the method of Hiscox and Israelstam (1979) was used. Dried, weighed algal samples on a dried filter membrane was placed in a 100 ml beaker and extracted in 7 ml of dimethyl sulfoxide (DMSO). The chlorophyll-DMSO filtrate was placed in an oven at 65 C for 3 h. After heating, the filtrate was removed from the oven, and more DMSO was added to each sample to bring the volume up to 10 ml. The chlorophyll-DMSO solution was transferred to cuvettes, placed in a dual wavelength spectrophotometer (Shimatzu-Model UV-160) and absorbance determined at 663 and 645 nm.

Microscopic examination of algal cultures: After incubation of the algal cultures, samples were removed and subjected to scanning and light microscopy. For light microscopy, samples were water-mounted. For electronic microscopy, samples were observed under a Cambridge 250 SEM at an accelerating voltage of 15 kV following fixation (Galvett, 1974; Wergin and Stone 1981).

RESULTS

Three levels of NCMC concentration (12, 24, and 39 nM) inhibited growth of Anabaena sp. as determined by reduction of algal biomass and chlorophyll production (Table 1). The NCMC produced growth inhibition during the first 6 days of incubation. Greater inhibition was observed at day 2 than at day 6 and no inhibition was observed at 9 days. Different patterns of pH changes were noted between chitosan-treated cultures and controls, although both type...