*Technical note*

# Control of *Microcystis aeruginosa* TH01109 with batangas mandarin skin and dwarf banana peel

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## Abstract

We studied the inhibitory effects of batangas mandarin skin and dwarf banana peel on *Microcystis aeruginosa*. In laboratory assays, algal growth was significantly inhibited by the addition of mandarin skin extract (0.1% w/v). When the concentration of mandarin skin increased to 0.5% (w/v), no algal growth was detected, whereas in the presence of banana peel extract (0.5%, w/v) the algal biomass was only slightly suppressed. The results show that mandarin skin has higher anti-algal activity than banana peel. Fresh unprocessed mandarin skin and banana peel showed very effective anti-algal activity. Pre-treatment was not required for their anti-algal activity. It is possible that mandarin skin and banana peel might be effective material to control harmful algal blooms.

**Keywords**: anti-algal activity, batangas mandarin skin, dwarf banana peel, inhibitors, *Microcystis aeruginosa*, algal bloom

## Introduction

The occurrence of harmful algal blooms in eutrophic water bodies is a worldwide problem. The production and release of a range of cyanotoxins is often associated with algal blooms (Codd et al., 1989; Codd, 1995). Of the toxic cyanobacterial genera, *Microcystis* is the most common and cosmopolitan genus. The most commonly reported cyanotoxins are hepatotoxins, the largest group being heptapeptides, known as microcystins. Via many exposure routes these toxins present human and animal health hazards (Turner et al., 1990; Bell and Codd, 1994; Falconer, 1996, 1997; Frazic et al., 1998). The deaths of more than 50 haemodialysis patients in Brazil have been attributed to exposure to microcystins in dialysis water (Jochimsen et al., 1998; Pouria et al., 1998). In China, it has become evident over recent times that the number of reports of toxic algal blooms in potable waters has increased. However, the mechanical removal of algal scum is energy and time consuming, and thus impractical. Also, the chemical treatment is undesirable in potable water supplies (Ball et al., 2001). Therefore, environmentally sound, economically favourable and effective methods are required to remove these harmful algae.

Decomposed barley straw has been reported to have algal-inhibiting properties (Welch et al., 1990). A number of other material have also been found to be anti-algal such as brown-rotted wood (Pillinger et al., 1995) and some leaf litters, in particular oak leaves (Ridge et al., 1995). The use of both barley straw and leaf litters to control algal growth depends on the inhibitors that they generate during aerobic decomposition in water (Gibson et al., 1990; Ridge et al., 1995). These inhibitors are derived from oxidised polyphenolics, which originate mainly from lignin (Pillinger et al., 1994, 1995; Ridge and Pillinger, 1996). Although barley straw has been shown to be a useful alternative to physical or chemical treatment for the prevention of algal blooms (Welch et al., 1990; Barrett et al., 1996; Ridge and Pillinger, 1996), the use of barley straw needs considerable management effort, and the long-term ecological safety of its use is unknown (Ridge et al., 1999). Furthermore, straw bales used to suppress algal growth may interfere with water traffic and fishing, and it is difficult to dispose of useless straw that has ceased to be anti-algal. Further study by Ball et al. (2001) indicated that decomposed barley straw extract also showed anti-algal capability. It seems that this straw extract could solve some of the problems caused by straw bales.

However, Martin and Ridge (1999) reported the different sensitivities of algal species to barley straw inhibitors, so it is possible that some harmful algae might increase if barley straw were used continuously for several years. It might be better to use several different materials at intervals for controlling harmful blooms. This led us to search for more effective and environmentally sound materials to inhibit unwanted algae.

The aim of this study was to investigate algal growth inhibitory effects of batangas mandarin skin and dwarf banana peel, to assess whether these could be novel anti-algal materials and to establish whether the use of mandarin skin or banana peel would prove a reliable and safe measure to control unwanted algae. To our knowledge, this is the first report to study the anti-algal effects of mandarin skin and banana peel.

## Materials and methods

### Materials

*Microcystis aeruginosa* TH01109 was originally isolated from Lake Taihu, Wuxi, Jiangsu, China and microscopically identified using the method described by Smith (1920) and Hu (1980). Cultures were maintained in BG-11 medium (Allen and Stanier, 1968).

Batangas mandarin (*Citrus reticulata* Blanco) and dwarf banana (*Musa cavendishii* Lamb) were obtained from Fujian, China. Mandarin skin and banana peel were washed with tap water and distilled water, and then dried in air.

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### Preparation of batangas mandarin skin and dwarf banana peel extracts

Extracts were prepared according to the method described by Ball et al. (2001), slightly modified. Fresh mandarin skin (50 g) was cut into pieces < 5 mm x 5 mm. After blending, an aliquot (10 g) of these pieces was boiled in 100 mℓ of distilled water for 2 h. Following cooling, the solution was filtered through glass fibre paper (Whatman GF/C), and the filtrate volume was adjusted to 100 mℓ. The banana peel extract was prepared in the same way.

### Assessment of the anti-algal activity of batangas mandarin skin and dwarf banana peel extracts

The anti-algal activity of mandarin skin and banana peel extracts was examined by the addition of the mandarin skin and banana peel extracts to BG-11 medium in 100 mℓ conical flasks. The final volume of bioassay is 75 mℓ. After the addition of the extract, the medium was autoclaved. Following cooling, the medium was inoculated with 1 mℓ of *M. aeruginosa* culture (in exponential growth phase) for each flask. All flasks were placed onto a shelf in a culture room at 22°C, illuminated in a 12 h/12 h light-dark cycle with fluorescent tubes in a light intensity of 5 000 lux, and shaken twice daily. For each test 2 mℓ of the culture per flask was removed 0, 6, 12, 19, 26 d after inoculation.

### Assessment of the anti-algal activity of autoclaved batangas mandarin skin and dwarf banana peel

Fresh mandarin skin or banana peel (0.075 g, < 5 mm x 5 mm cut) was added to BG-11 medium (75 mℓ) in each flask (100 mℓ), and the medium was then autoclaved. After cooling, the medium was inoculated with 1 mℓ of *M. aeruginosa* culture (in exponential growth phase) for each flask. The flasks were incubated for up to 26 d on the same shelf as mentioned above, and shaken twice daily.

### Assessment of the anti-algal activity of fresh batangas mandarin skin and dwarf banana peel

Fresh mandarin skin and banana peel were disinfected in 0.1% $HgCl_2$ solution for 10 min and then washed four times with sterile water. After the sterile mandarin skin or banana peel (0.075 g, <5 mm x 5 mm cut) was added to the autoclaved BG-11 medium (75 mℓ) in each flask (100 mℓ), the medium was inoculated with 1 mℓ of *M. aeruginosa* culture (in exponential growth phase) for each flask. The flasks were incubated for up to 26 d on the shelf and shaken twice daily.

### Biomass estimation

Algal growth was quantified by cell counting on a hemacytometer. Experiments were carried out on a range of doses of mandarin skin and banana peel, using four replicate flasks for each dose.

### Determination of total phenolics and tannin contents

Total phenolics and tannin contents were determined by the slightly modified methods described by Price and Butler (1977) and Wang et al. (1998). Fresh mandarin skin (1 g) or banana peel (1 g) was finely chopped, ground to a slurry with a mortar and pestle with 5 mℓ of absolute methanol and shaken on a reciprocating shaker for 30 min. An aliquot was centrifuged at 13 000 r/min for 15 min. The supernatant was diluted 100 times with distilled water mixed with

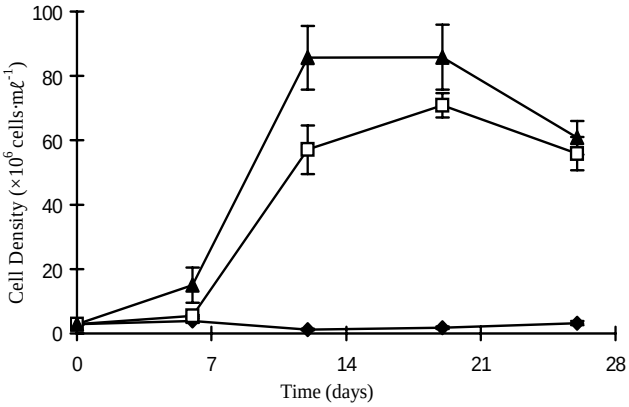


***Figure 1***

*The growth of algal biomass, as determined by cell density (cells/mℓ) in BG-11 medium (control, ▲) and the effects of the addition of batangas mandarin skin extract (◆) and dwarf banana peel extract (□) (both 0.5% w/v) on algal growth over 26 d. Data points represent means of four replicates ±S.D.*

3 mℓ of 0.1M $FeCl_3$ in 0.1N HCl for 3 min, followed by the timed addition of 3 mℓ of 0.008 M $K_3Fe(CN)_6$. The absorbance was read after 10 min at 720 nm on a spectrophotometer. For total phenolics a blank of identical composition, but replacing the sample extract with absolute methanol, was analysed. A duplicate extraction in 0.2 M NaCl was conducted and this 'NaCl blank' was used for determining tannin content. The standard curve for total phenolics and tannin was prepared with catechin, and the results were expressed in terms of catechin equivalents.

## Results

### Comparison of the inhibitory properties of batangas mandarin skin and dwarf banana peel extracts

Figure 1 showed that mandarin skin and banana peel had very different inhibitory effect on *M. aeruginosa* growth. In the presence of mandarin skin extract (0.5%, w/v, final concentration of mandarin skin, equal to 0.095% dry w/v) *M. aeruginosa* was completely inhibited over 26 d. The banana peel extract (0.5%, w/v, final concentration of banana peel, equal to 0.054% dry w/v) inhibited the growth of *M. aeruginosa* to some extent. By the addition of banana peel, the biomass was 36.7%, 66.7%, 82.5%, 91.8% of control after 6, 12, 19 and 26 d incubation respectively, indicating that the inhibitory effect of banana peel on *M. aeruginosa* decreased gradually with the incubation. This result is probably attributable to the consumption of the inhibitor, banana peel.

It was suggested earlier that phenolic compounds play an important role in the inhibition of algae (Ridge and Pillinger, 1996; Nakai et al., 2001). As one of the important phenolics in edible fruits, tannin was also thought to be algicidal (Hussein-Ayoub and Yankov, 1985; Ridge et al., 1995). Our results (Table 1) show that the mandarin skin, with more powerful anti-algal property, has significantly higher content of total phenolics and tannin than the banana peel.

### Effect of different concentration of batangas mandarin skin and dwarf banana peel extracts on the growth of *M. aeruginosa*

The effect of different concentration of mandarin skin and banana peel extracts on *M. aeruginosa* is shown in Table 2. Less than

**TABLE 1**
**Total phenolics and tannin levels of batangas mandarin skin and dwarf banana peel in terms of catechin equivalents on the basis of fresh and dry mass. Values are the means±S.D. (n=4). (Significant differences between mandarin skin and banana peel are shown as ** p < 0.001)**

| | Total phenolics | | Tannin | |
|---|---|---|---|---|
| | (mg/kg FW) | (mg/kg DW) | (mg/kg FW) | (mg/kg DW) |
| Banana peel | 387.34±7.65 | 3606.55±71.19 | 202.59±8.71 | 1886.32±81.05 |
| Mandarin skin | 964.11±22.98** | 5093.05±121.40** | 462.09±25.14** | 2441.06±132.82** |

**TABLE 2**
**Effect of the addition of batangas mandarin skin and dwarf banana peel extracts on growth of *M. aeruginosa* in BG-11 medium at 22°C over 19 d. Values are the means±S.D. (n=4). (Significant differences from the control are shown as ** p < 0.001)**

| Concentration of extract (%) (w/v) | Cell density (×10⁶ cells/mℓ) | |
|---|---|---|
| | Mandarin skin | Banana peel |
| 0(control) | 85.83±10.10 | 85.83±10.10 |
| 0.002% | 93.13±16.23 | 94.17±6.29 |
| 0.01% | 87.50±2.50 | 86.67±1.44 |
| 0.1% | 35.83±5.20** | 84.17±3.82 |
| 0.5% | 1.83±0.38** | 70.83±3.82 |

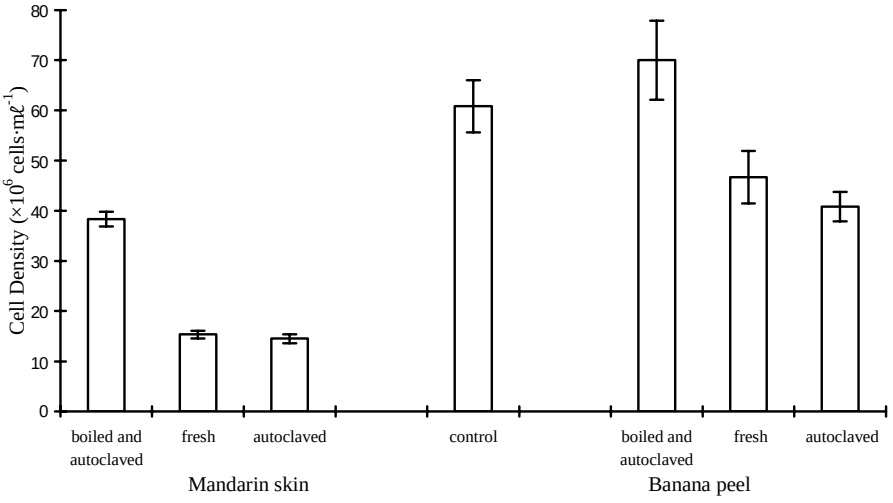


***Figure 2***
*Effects on* M. aeruginosa *growth of boiled and autoclaved (autoclaved extract), autoclaved, and fresh batangas mandarin skin and dwarf banana peel (all 0.1%, w/v) in BG-11 medium at 22°C over 26 d. Columns represent means of four replicates±S.D.*

0.01% of mandarin skin and banana peel had no inhibitory effect and resulted in a small additional growth of *M. aeruginosa* compared with the control flask, which contained no extract. When the concentration of mandarin skin was higher than 0.1%, the inhibition was significant ($p<0.001$). However, only after adding 0.5% of banana peel was the inhibition significant ($p<0.05$). The results (see Fig. 1, Table 2) show that *M. aeruginosa* is only slightly susceptible to banana peel in contrast with mandarin skin.

### Comparison of the anti-algal effects of batangas mandarin skin and dwarf banana peel in different pre-treatments

The effects of different pre-treatments of batangas mandarin skin and dwarf banana peel on *M. aeruginosa* were examined (Fig. 2). The results showed that in all cases, the inhibition for mandarin skin was significant ($p<0.01$), whereas the presence of autoclaved extract derived from banana peel increased algal biomass after 26 d incubation. In particular, both fresh or autoclaved mandarin skin and banana peel had stronger anti-algal activity than their autoclaved extracts, and the differences are significant ($p<0.001$ for mandarin skin; $p<0.01$ for banana peel).

## Discussion

The anti-algal activity of plant litter is attributed to litter-derived inhibitors (Gibson et al., 1990; Pillinger et al., 1994). With the addition of autoclaved banana peel extract (0.1%), the biomass was 46.7%, 86.1%, 98.1%, 115.1% of control after 6 d, 12 d, 19 d and 26 d, respectively (data not shown). Further, in contrast to the inhibition of *M. aeruginosa* by higher concentration of mandarin skin and banana peel extracts, lower concentrations of extracts resulted in slightly higher biomass than the control (Table 2). The results probably reflect a balance between the release of inhibitors and nutrients in the case of the addition of mandarin skin and banana peel. When the inhibitors take the lead, the inhibitory effect occurs.

Fresh unprocessed mandarin skin and banana peel showed very effective anti-algal activity (see Fig. 2). This is different from previous reports, which suggested that barley straw (Martin and Ridge, 1999), brown-rotten wood (Pillinger et al., 1995), deciduous leaf litter and coniferous leaf litter (Ridge et al., 1995) require pre-treatment or oxidising conditions for inhibitory activity (Ridge et al., 1999). Our laboratory studies suggest that without boiling, autoclaving, or grinding to create artificial

FPOM (fine particulate matter) (Ridge et al., 1999), mandarin skin and banana peel still show inhibitory effect on *M. aeruginosa*. *M. aeruginosa* is a commonly occurring blue-green algal that often forms water bloom and produces microcystin in China. By mouse bioassay the cyanobacterium *M. aeruginosa* isolated from Lake Taihu, the third largest freshwater lake in China, produces hepatotoxin (data not shown). The laboratory studies presented here indicate that without any pre-treatment mandarin skin and banana peel can be effective anti-algal factors. Therefore, water treated with mandarin skin and banana peel through simple soaking might be effective to control this harmful species. This may have some implications for water management. Nevertheless, further work is desirable to determine whether the inhibitory action could be extrapolated into the field and that no adverse effects of using mandarin skin and banana peel occur.

## Conclusions

Our laboratory studies show that batangas mandarin skin and dwarf banana peel inhibit the growth of *M. aeruginosa* isolated from Lake Taihu of China. Mandarin skin shows more powerful anti-algal properties than banana peel. Fresh unprocessed mandarin skin and banana peel show very effective anti-algal activity. Pre-treatment is not indispensable for their inhibitory activity. These results indicate that mandarin skin and banana peel might be potential anti-algal materials. However, before mandarin skin and banana peel are applied in water management it has to be examined whether these would have any adverse effects on other living organisms.

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