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Algicidal Effects of Korean Oak Trees against the Cyanobacterium *Microcystis aeruginosa*

Park, Myung-Hwan[1], Baik-Ho Kim[2], Myung-Soo Han[1,2], Chi-Yong Ahn[3], Byung-Dae Yoon[3] and Hee-Mock Oh[3,*]

([1]Department of Environmental Science, Hanyang University, Seoul 133-791, Korea
[2]Department of Life Science, Hanyang University, Seoul 133-791, Korea
[3]Environmental Biotechnology Laboratory, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, Korea)

In an effort to identify a new environment-friendly algicide, we examined the ability of extracts from the leaves and stems of nine Korean oak tree species to inhibit growth of the bloom-forming cyanobacterium, *Microcystis aeruginosa*. At a concentration of 100 mg L^{-1}, five of the oak tree extracts (QAT-L, QAT-S, QAS-L, QGI-S, and QSA-L) decreased the cell density of *M. aeruginosa* by over 90% for 7 days. At a concentration of 20 mg L^{-1}, the same five extracts inhibited the growth of *M. aeruginosa* by approximately 50%. The minimum concentration of oak tree extracts required for effective inhibition of *M. aeruginosa* (20 mg L^{-1}) is comparable to that of the known algicide, tannic acid (17 mg L^{-1}), which is thought to be one of the main active ingredients in the oak tree extract. These findings suggest that oak extracts may be useful as an environment-friendly algicide to control the bloom-forming cyanobacterium, *M. aeruginosa*, in eutrophic waters.

Key words : Algal inhibition, Allelochemical, *Microcystis aeruginosa*, Oak extracts, Tannic acid, Cyanobacteria

INTRODUCTION

The cyanobacterium, *Microcystis aeruginosa*, is one of the most common bloom-forming phytoplankton species in eutrophic lakes and reservoirs worldwide (Oh *et al*., 2000), and has been associated with harmful effects on animals and humans (Skulberg *et al*., 1984; Song *et al*., 1998), including allergies, irritation reactions, gastroenteritis, liver diseases and tumors (An and Carmichael, 1994; Bell and Codd, 1994; Dawson, 1998). Cyanobacterial blooms also cause water problems, such as foul odors, decreased aesthetic value, deterioration of water quality, and deoxygenation (Sigee *et al*., 1999). However, although the physiology and ecology of *M. aeruginosa* have been extensively studied in terms of bloom mechanisms and toxicity to aquatic organisms, the growth inhibition or bioremediation of *M. aeruginosa* blooms is less well understood (Reynolds, 1984; Paerl, 1988; Zohary and Robarts, 1989; Manage *et al*., 2001).

Allelochemicals are secondary metabolites involving in allelopathy produced by higher plants, which have been shown to inhibit or stimulate the growth of microorganisms in aquatic ecosystems (Pillinger *et al*., 1994; Everall and Lees, 1997; Nakai *et al*., 2000, 2001; Ebana *et al*., 2001). These Fchemicals originate from various plant tissues (leaves, flowers, fruits, stems, and roots), and they include mainly phenolic acids, flavo-

* Corresponding Author: Tel: +82-42-860-4321, Fax: +82-42-860-4594, E-mail: heemock@kribb.re.kr

noids, terpenoids, steroids, alkaloids and organic cyanide (Whittaker and Feeny, 1971). Of these, phenolic compounds such as caffeic acid, gallic acid, syringic acid and tannic acid, have been widely applied as biocides for phytoplankton control (Pillinger *et al*., 1994; Everall and Lees, 1997; Nakai *et al*., 2000, 2001), and tannic acid is recognized as both a bactericide and an algicide (Hussein-Ayoub and Yankov, 1985; Lee and Shin, 1991). Compared to other higher plants, the oak tree contains relatively high levels of tannic acid. As the oak tree accounts for approximately 27% of the total national forest in Korea (Song *et al*., 2002), it is interesting to speculate that this higher plant could yield environment-friendly biological controls for use in Korea. However, no previous report has examined the feasibility of using oak tree extracts for controlling harmful algal blooms.

Here, we examined the abilities of extracts from nine species of Korean oak tree (*Castanopsis cuspidata* var. *sieboldii*, *Quercus acuta*, *Q. acutissima*, *Q. aliena*, *Q. dentata*, *Q. gilva*, *Q. glauca*, *Q. salicina*, and *Q. serrata*) to inhibit the growth of the cyanobacterium, *M. aeruginosa*, and compared their effective concentrations to that of tannic acid.

MATERIALS AND METHODS

1. Algal strain and culture conditions

The cyanobacterium, *Microcystis, aeruginosa*, strain UTEX 2388 was obtained from the Culture Collection of Algae at the University of Texas (Austin, TX, USA) and grown in Allen medium (Allen, 1968) [1,500 mg $NaNO_3$, 39 mg K_2HPO_4, 75 mg $MgSO_4 \cdot 7H_2O$, 21 mg Na_2CO_3, 27 mg $CaCl_2$, 58 mg $Na_2SiO_3 \cdot 9H_2O$, 1 mg EDTA, 6 mg citric acid, 6 mg ferric citrate, 2.86 mg H_3BO_3, 1.81 mg $MnCl_2 \cdot 4H_2O$, 0.22 mg $ZnSO_4 \cdot 7H_2O$, 0.39 mg $Na_2MoO_4 \cdot 2H_2O$, 0.08 mg $CuSO_4 \cdot 5H_2O$ and 0.05 mg $Co(NO_3)_2 \cdot 6H_2O$ in 1,000 mL of distilled water, adjusted to pH 7.8] at 28°C in a shaking incubator with a 14-h light/10-h dark cycle (100 μE m^{-2} s^{-1} illumination provided by cool-white fluorescent lamps). *Microcystis* cells were batch-cultured, and every two weeks, 10 mL samples of each culture were inoculated into 90 mL of fresh medium for use as a seed culture.

2. Preparation of oak tree extracts

Extracts from nine representative Korean oak trees were examined for their abilities to inhibit algal growth. Table 1 summarizes the species and plant tissues from which each extract was obtained. Extracts were prepared as described by Suzuki *et al*. (1998), with some modification. Briefly, strips of plant material (100 g) were mixed with methanol (1,500 mL) and then sonicated for 10 min at 50°C. The solution was filtered through a GF/C filter (No. 1822 047 Whatman, England) and evaporated. The resulting extract was concentrated to 1% (dry weight basis) and stored in a freezer (−10°C) until use.

3. Inhibition of algal growth by oak extracts and tannic acid

The seventeen different methanol extracts from the leaves and/or stems of nine different Korean oak tree species were adjusted to 100 mg L^{-1} (w/v) and mixed with 100 mL of algal culture in 250 mL triangle flasks. Water from the Daechung Reservoir was used as the culture medium after filtration through a GF/C filter (No. 1822 047, Whatman, England). Changes in the cell density of *M. aeruginosa* were measured with a Coulter counter (Coulter Z1, Coulter Corp., USA). Five extracts were found to inhibit algal growth by more than 90% when applied at 100 mg L^{-1}. We then examined the specific activity of these five extracts at 10, 20 and 50 mg L^{-1}, using the above conditions. For comparison, we also examined the inhibitory ability of different concentrations (1, 5 and 10 μM, corresponding to 1.7, 8.5 and 17 mg L^{-1}, respectively) of experimental-grade tannic acid (Sigma, USA), a major phenolic compound in the extracts.

4. Data analysis

Algal inhibition activity was calculated using the modified equation of Suzuki *et al*. (1998) and Chung *et al*. (2001):

Algal Inhibition Activity (%)
= [(Control − Treatment)/Control] × 100

RESULTS AND DISCUSSION

Figure 1 shows the effects of the various oak tree extracts on growth of the cyanobacterium,

M. aeruginosa. Out of 17 different methanol extracts, five extracts (QAT–L, QAT–S, QAS–L, QGI–S and QSA–L; see Table 1 for abbreviations) were found to decrease the cell density of *M. aeruginosa* by over 90% on day 7. Seven other extracts (CC–L, CC–S, QAI–LS, QAS–S, QGL–L, QSA–S and QSE–S) decreased the growth of *M. aeruginosa* by approximately 50% compared to untreated control over the same period. Table 2 shows the effects of various concentrations of the five most potent extracts on the growth of *M. aeruginosa*. At 50 mg L^{-1}, QAT–L, QAS–L and QSA–L strongly inhibited algal growth by more than 90%, while QAT–S and QGI–S moderately inhibited growth by 63% and 78%. At 20 mg L^{-1}, all five extracts inhibited the growth of *M. aeruginosa* by approximately 50%. At 10 mg L^{-1}, only QSA–L (43%) and QAT–L (25%) inhibited the growth of *M. aeruginosa*, while the other three extracts either had little effect or stimulated algal growth. These results indicate that the minimum level of *Quercus* extract needed for effective inhibition of algal growth is 20 mg L^{-1}.

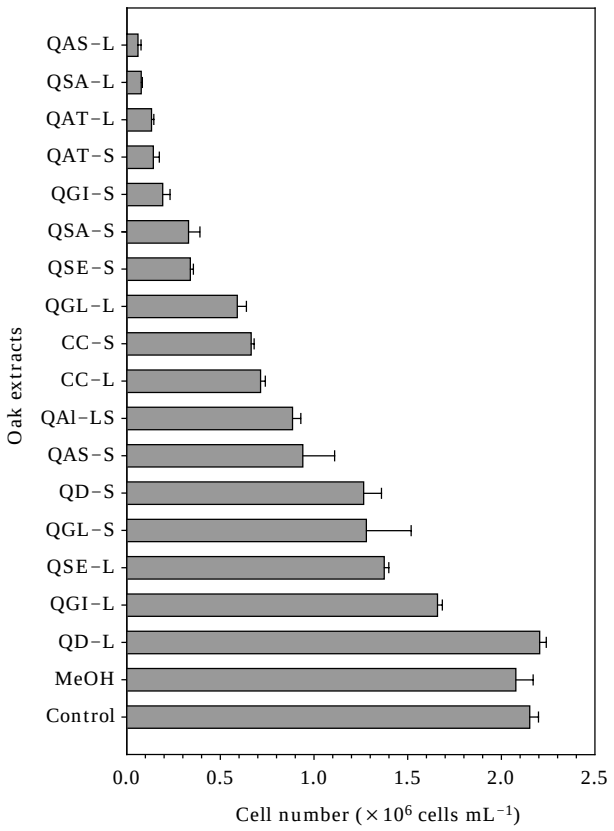

Fig. 1. Effects of plant extracts (100 mg L^{-1}) on the growth of *M. aeruginosa* (cell number) in Daechung Reservoir. Results indicate the means ± SE (n = 3).

It is likely that at least some of this algicidal activity is due to the presence of tannins in the oak tree extracts. Tannins, which are known algicides (Hussein–Ayoub and Yankov, 1985) commonly found as byproducts of oxidative degradation in the oak tree (Ridge *et al.*, 1999), are thought to inhibit algal growth by altering respiration, calcium and potassium uptake, photosynthesis, and membrane permeability (Duke, 1986). Previous studies have shown that the tannin contents in various oak trees vary from 5 to 100 g kg^{-1} (Table 3), with the genus *Quercus* showing relatively high levels (Ridge *et al.*, 1999). In addition to species–specific differences, tannic acid levels in oak trees also vary seasonally, showing higher levels in the fall (September) versus spring (April) (Feeny and Bostock, 1968). We next compared the effective algicidal concentrations required of the oak tree extracts with that of tannic acid. Figure 2 shows the anti–algal effects of various concentrations of experimental–grade tannic acid on the growth of *M. aeruginosa* after 2 and 5 days. Our results revealed that the minimum concentration of tannic required to inhibit growth of *M. aeruginosa* was 10 μM (17 mg L^{-1}), whereas concentrations of 1 and 5 μM (1.7 and 8.5 mg L^{-1}) enhanced algal growth by as much as 150%. Previous studies have shown that the growth of another cyanobacterium, *Anabaena* sp., was ef-

Table 1. Methanol extracts from *Quercus* and *Castanopsis* in Korean oak.

Symbol	Scientific name	Parts
CC–L	*Castanopsis cuspidata* var. *sieboldii*	Leaf
CC–S	*Castanopsis cuspidata* var. *sieboldii*	Stem
QAT–L	*Quercus acuta*	Leaf
QAT–S	*Quercus acuta*	Stem
QAS–L	*Quercus acutissima*	Leaf
QAS–S	*Quercus acutissima*	Stem
QAl–LS	*Quercus aliena*	Leaf, stem
QD–L	*Quercus dentata*	Leaf
QD–S	*Quercus dentata*	Stem
QGI–L	*Quercus gilva*	Leaf
QGI–S	*Quercus gilva*	Stem
QGL–L	*Quercus glauca*	Leaf
QGL–S	*Quercus glauca*	Stem
QSA–L	*Quercus salicina*	Leaf
QSA–S	*Quercus salicina*	Stem
QSE–L	*Quercus serrata*	Leaf
QSE–S	*Quercus serrata*	Stem

Table 2. Effects of various concentrations of oak extracts on the growth of *M. aeruginosa*.

Oak extract	10 mg L^{-1}		20 mg L^{-1}		50 mg L^{-1}	
	Cell number (10^6 cells mL^{-1})[a]	%[b]	Cell number (10^6 cells mL^{-1})[a]	%[b]	Cell number (10^6 cells mL^{-1})[a]	%[b]
Control	1.30±0.09		1.30±0.09		1.30±0.09	
QSA-L	0.74±0.02	43	0.65±0.10	50	0.03±0.00	98
QAS-L	>1.30	-	0.29±0.00	78	0.12±0.00	91
QAT-L	0.98±0.03	25	0.34±0.02	74	0.12±0.00	91
QGI-S	>1.30	-	0.48±0.08	63	0.28±0.00	78
QAT-S	>1.30	-	0.54±0.19	58	0.48±0.01	63

QSA-L, *Quercus salicina* leaf extract; QAS-L, *Quercus acutissima* leaf extract; QAT-L, *Quercus acuta* leaf extract; QGI-S, *Quercus gilva* stem extract; QAT-S, *Quercus acuta* stem extract. [a]Values represent the means ± SE (n = 3). [b]Algal Inhibition Activity (%) = [(Control − Treatment)/Control] × 100

Table 3. Comparison of tannic acid contents from different oak species.

Oak species	Tannic acid content (g kg^{-1})	Part	References
Quercus robur	5 (April) 50 (September)	Leaf	Feeny and Bostock (1968)
Oak 1	80	Wood	Bianco and Savolainen (1997)
Oak 2	100	Wood	Bianco and Savolainen (1997)
Quercus branti	14.9	Leaf	Kamalak *et al.* (2004)
Quercus coccifera	42.0	Leaf	Kamalak *et al.* (2004)
Quercus cercis	47.9	Leaf	Kamalak *et al.* (2004)
Quercus libari	15.5	Leaf	Kamalak *et al.* (2004)
Quercus infectaria	22.4	Leaf	Kamalak *et al.* (2004)

fectively inhibited by 6 μM of tannic acid (Rice, 1984), and similar concentrations of tannin inhibited growth of the green algae, *Chlorella vulagris* (Pillinger *et al.*, 1994). In contrast, 100 μM of tannic acid was required to effectively inhibit the cyanobacterium, *Oscillatoria* cf. *chalybea*, and the green algae, *Selenastrum capricornutum* (Schrader *et al.*, 1998). These results indicate that the algicidal activities of tannic acid are target algae-specific, and that the effective concentration of tannic acid against the growth of *M. aeruginosa* (17 mg L^{-1}) was relatively low, and was similar to that of the oak tree extract (20 mg L^{-1}).

Numerous chemical agents and synthetic compounds (e.g. copper, chlorine, aluminum, calcium and potassium permanganate) are currently used to control algal blooms in lakes, reservoirs and ponds. However, these chemicals often induce secondary problems, such as the toxic effects of copper on carp (Karan *et al.*, 1998) and the release of phytotoxins that increase potential health risks in drinking water supplies (Lam *et al.*, 1995). In an effort to develop a more environment-friendly algicidal strategy, a number of studies have examined the abilities of various allelochemicals and plant extracts to inhibit the growth of phytoplankton species. For example, growth of *Microcystis*, *Spirogyra*, *Selenastrum*, *Chlorella* and *Ankistrodesmus* have been shown to be inhibited by barley straw (Gibson *et al.*, 1990; Welch *et al.*, 1990; Pillinger *et al.*, 1994; Brownlee *et al.*, 2003), and *Chlorella* may also be inhibited by oak, pine, willow, elm, birch, sycamore and rowan (Ridge and Pillinger, 1996; Ridge *et al.*, 1999). However, this is the first study to examine the ability of oak tree extracts to control the harmful cyanobacterium, *M. aeruginosa*.

The oak tree is an important endemic species in Korea (You *et al.*, 2001), where its distribution has gradually increased over time as a result of artificial planting and the aggressive natural succession of one species, *Q. mongolica* (You *et al.*, 1995). The oak accounts for −27% of the total tree biomass production in Korea (Song *et al.*, 2002), and of the 600,000 m^3 of annual lumbering in Korea (ca. 1999), −30% was oak (Korea Forest Service, 2000). The majority of smaller cuttings (<10 cm in diameter) are culled, meaning that −35% of oak is discharged as waste annually.

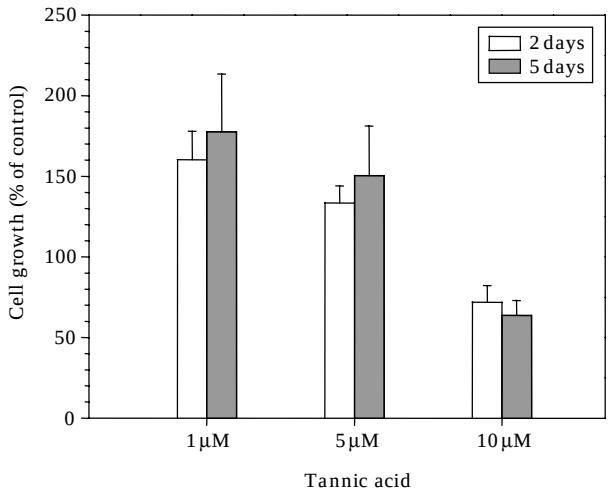

Fig. 2. Effects of various concentrations of tannic acid on the growth of *M. aeruginosa*. Initial concentrations of tannic acid were 1, 5, and 10 μM (1.7, 8.5, and 17 mg L^{-1}, respectively). Natural water was obtained from the Daechung Reservoir. Results indicate the means ± SE (n = 3).

Our finding that Korean oak tree extracts effectively inhibited the growth of *M. aeruginosa* suggests that this large mass of oak waste might be salvaged as an algicide.

Thus, we herein showed that oak tree extracts effectively inhibit growth of the cyanobacterium, *M. aeruginosa*, at relatively low concentrations (possibly because of synergistic action by multiple phenolic compounds). The application of oak extracts to control blooms of cyanobacteria such as *M. aeruginosa* has three major advantages: 1) oak extracts are easily dissociated by natural microorganisms (mainly bacteria), thus theoretically rendering them relatively nontoxic to the environment, 2) tannins do not affect the growth of other aquatic microorganisms (Street, 1979; Gary *et al*., 1983), and 3) the use of oak may minimize economic costs because it involves recycling of forestal waste. These findings suggest that additional studies are warranted to examine the effects of tannin or oak tree extracts in aquatic ecosystems, with the goal of developing a new strategy for ameliorating harmful algal blooms.

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＜**국문적요**＞

남조류 *Microcystis aeruginosa*에 대한 국내 참나무들의 살조 효과

박명환[1] · 김백호[2] · 한명수[1,2] · 안치용[3] · 윤병대[3] · 오희목[3,*]

([1]한양대학교 환경과학과, [2]한양대학교 생명과학과,
[3]한국생명공학연구원 환경생명공학연구실)

본 연구는 새로운 환경친화적 살조제를 탐색하기 위한 연구의 일환으로, 담수에서 조류 대발생 원인종 *Microcystis aeruginosa*를 대상으로 하여 국내에 자생하는 9가지 참나무 잎과 줄기로부터 추출한 물질의 조류생장 억제능력을 조사하였다. 추출물 농도 100 mg L^{-1} 첨가시, 5가지 참나무 추출물(QAT-L, QAT-S, QAS-L, QGI-S 및 QSA-L)은 *M. aeruginosa*의 7일 후 세포수를 90% 이상 감소시켰다. 20 mg L^{-1}의 경우에는 상기의 5가지 참나무 추출물에서 *M. aeruginosa*의 세포수가 약 50% 정도 감소하였다. *M. aeruginosa*를 효과적으로 제어하기 위한 참나무 추출물의 최소 농도는 20 mg L^{-1}로 조사되었다. 참나무의 주요 성분으로 알려져 있는 tannic acid는 17 mg L^{-1}의 농도에서 *M. aeruginosa*에 대한 억제효과를 나타냈다. 본 연구를 통해서 참나무 추출물은 부영양 수계에서 조류 대발생 원인종 *M. aeruginosa*를 제어하는 환경친화적 살조제로서 이용할 수 있을 것으로 판단된다.