A review of allelopathy on microalgae

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Abstract

Algal blooms have severe impacts on the utilization of water resources. The discovery of allelopathy provides a new dimension to solving this problem due to its high efficiency, safety and economy. Allelopathy can suppress the growth of microalgae by impairing the structure, photosynthesis and enzyme activity of algal cells. In the current work, we first demonstrate the allelopathy and allelochemicals derived from both plants and algae. We then expound the potential mechanisms of allelopathy on microalgae. Next, the potential application of allelochemicals in water environment is proposed. Finally, the key challenge and future perspective are presented.

INTRODUCTION

Harmful algal blooms (HABs) have frequently emerged in recent times due to anthropogenic eutrophication and climate change [1–3]. This has negative influences on the utilization of water resources (e.g. fisheries, water supplies and recruitment) [4]. The release of toxic secondary metabolites derived from microalgae not only does harm to human health [5], but also endangers other organisms (i.e. submerged macrophytes [6], animals [7], phytoplankton [8]). The economic loss caused by a serious HAB event is estimated to exceed millions of U.S. dollars [9]. A series of serious environmental and economic problems induced by HABs and their control have aroused great social concern [10–12]. Although various methods (e.g. copper sulfate and potassium permanganate) have been proposed to control harmful algal growth, secondary pollution, high cost or impracticability constrain their application [13, 14]. Therefore, it is imperative to investigate environmentally friendly algicides characterized by high efficiency, safety and economy. The discovery of allelopathy provides a new dimension to solving this problem [15, 16].

Allelopathy (i.e. inhibition) is commonly defined as the process involving chemical compounds released into the surrounding medium that have adverse effects, either directly or indirectly, on the growth of microorganisms [17, 18]. These chemical compounds, called allelochemicals, will be not only biodegradable but also cause less pollution than traditional herbicides, due to their natural origin [19]. This phenomenon was first discovered in terrestrial ecosystems, following which allelopathy between aquatic plants has gained considerable attention. In recent years, research on the allelopathy of phytoplankton has gradually increased. Generally speaking, allelochemicals are released by either plants or algae. The objectives of this review are to summarize current research on allelopathic effects on the growth of microalgae, and to expound the inhibition mechanism as well as potential applications in order to provide theoretical support for controlling algal blooms.

EXISTENCE OF ALLELOPATHY

Plant-derived allelopathy

Various factors, including algal species, their growth stages and initial concentration, have been investigated and shown to influence the effects of allelochemicals. For instance, the allelochemical ethyl 2-methylacetoacetate (EMA) isolated from Phragmites communis Tris showed strong inhibitory activity on the growth of Microcystis aeruginosa and Chlorella pyrenoidosa, the effective concentrations 50 % algal inhibition being 0.79 and 0.49 mg l−1, respectively. However, this compound had no inhibitory effect on Chlorella vulgaris [4]. Over the same cultivation period, EC50 increased with increase in initial algal density [20]. Allelochemical type, dosage and frequency and synergy affect the inhibition of algae. Many allelochemicals have been isolated and identified, including phenolic acids, fatty acids,
tannic acid, lactones, terpenoids, alkaloids, flavonoids and sulfides [21–25]. It is common knowledge that plants can release more than one type of allelochemical. Gao et al. [26, 27] studied the combined effects of allelochemicals on the growth of M. aeruginosa, and found that the synergistic effect was affected by mixing ratio and the activity of individual allelochemicals.

The release of allelochemicals from plants is a continuous process [28]. The concentration of allelochemicals in natural water bodies is usually lower than their effective concentration when used experimentally to uncover the mechanism of inhibition [29]. Therefore, daily and continuous addition of these chemicals is worthy of further study. Gao et al. [30] provided evidence that the inhibitory effect of N-phenyl-1-naphthylamine on M. aeruginosa was prolonged in a low-dosage, repeated-exposure pattern compared to that in a high-dosage, single-exposure pattern. The growth of M. aeruginosa was effectively inhibited only when the daily dose of pyrogallol reached 0.5 mg l$^{-1}$ [29]. With the discovery of ever more allelochemicals, they can now be distinguished by cluster analysis while the relationship between their structure and algal inhibition efficiency has also been researched. Nakai et al. [16] indicated that polyphenols with the ‘orthob’ and/or ‘para’ hydroxy groups had stronger inhibitory effects on M. aeruginosa compared to those with meta-position hydroxy groups. With regard to the algal inhibitory effect of fatty acids, the greater the number of unsaturated linkages and the shorter the carbon chain, the greater the inhibitory effect. Fatty acids with an odd number of carbon atoms have better algal inhibitory effects than those with an even number [31].

The inhibitory effect of allelochemicals on algae has been studied under different environmental factors, including pH, temperature and light. For instance, Nakai et al. [32] demonstrated that the five phenolic acids and three fatty acids isolated from Myriophyllum spicatum had a better inhibitory effect on M. aeruginosa under weak lighting than those under increased illumination.

**Algae-derived allelopathy**

Inderjit and Dakshini were the first to define the allelopathy of algae, believing that substances secreted by algae could affect their own growth of that of other algae nearby [33]. Chlorellin, released by C. vulgaris, significantly inhibited the growth of Pseudokirchneriella subcapitata [34]. β-Lonone, a secondary metabolite secreted by Tychonema bourrelyi, is a type of allelochemical with allelopathic effects on M. aeruginosa [35]. Strains of Cylindrospermopsis raciborskii can produce unknown bioactive compounds that can imitate the actions of cylindrospermopsin to induce toxic effects as demonstrated by the inhibition in growth of M. aeruginosa [36]. The allelochemicals released by M. aeruginosa inhibit the growth of C. pyrenoidosa and Cyclotella meneghiniana [37]. Fischereella sp. strain 52-1 inhibited the growth of all tested green algae and cyanobacteria isolated from south and central Florida. In addition, photosynthesis of Chlamydomonas sp. was inhibited by crude lipophilic extracts from strain 52-1 [38]. Similar evidence was found by Gross [39]. Oscillatoria sp. produced complex secondary metabolites that inhibited the growth of C. vulgaris. These metabolites were isolated and structurally characterized as cyclic peptides containing several unusually modified amino acids [40]. C18 fatty acids, released by C. vulgaris, had an allelopathic inhibitory effect on Pseudokirchneriella subcapitata [41]. A wide variety of recent studies have demonstrated that secondary metabolites secreted by algae can adversely affect various organisms. These allelochemicals include mostly polyunsaturated fatty acids and their derivatives, alkaloids and microcystin [42].

**MECHANISM OF ALLELOPATHY**

**Effects of allelopathy on photosynthesis**

Photosynthesis is one of the most important physiological processes in algal growth. The algal photosystem consists of photosystem I (PSI), photosystem II (PSII) and an electron transfer body [43]. Under the action of allelochemicals, algal photosynthetic pigments and certain protein complexes will be affected. For instance, phycobilisomes were destroyed and chlorophyll content decreased due to the inhibition of chlorophyll synthesis or/and accelerated degradation of chlorophyll [44, 45]. In addition, allelochemicals can block electron transfer in algal photosynthetic systems and further influence the synthesis of adenosine triphosphate (ATP). Zhu et al. [46] found that the PS IIand whole-electron transport chain activities of M. aeruginosa were reduced by exposure to pyrogallic acid (PA) and gallic acid (GA). Previous researches have shown that certain allelochemicals directly inhibit electron transport by specifically binding to sites in PS II. Cyanobactrin, from Scytomena, is associated with the oxidizing aspect of the Qb (secondary bound plastoquinone) electron acceptor [47]. Fischerellin A, produced by Fischerella muscicola, probably acts at several sites in PS II [48].

**Effects of allelopathy on cell structure**

The cell membrane is an important barrier in the exchange of matter and energy between the cell and its external environment. Once the cellular membrane is damaged, not only the structure and function of organelles will be affected, but also various substances in the aqueous environment will enter the cell and intracellular components will be expelled. Research shows that the main fatty acids in the cell membranes of certain algae will be oxidized and that the degree of unsaturation will increase under the action of allelochemicals, which will enhance cell membrane fluidity and reduce its selectivity permeability [49]. Hu et al. [44, 50] found that exposure to EMA increased the levels of unsaturated fatty acids in the cell membranes of C. pyrenoidosa and M. aeruginosa, resulting in the extravasation of metal ions including K$^+$ and Mg$^{2+}$ into cells. However, this had no significant effects on the relative content of fatty acids in the cell membrane of C. vulgaris. Moreover, when exposed to a certain concentration of allelochemicals, cell shape will change: the protoplast will shrink and separate from the cell wall,
nucleus will condense and the structure of chloroplasts will be disrupted [45, 51].

**Effects of allelopathy on enzyme activities of algal cells**

Enzyme function plays a vital role in the organism because its functional changes can directly or indirectly affect the growth and physiological characteristics of the organism. Hong et al. [52] found that the activities of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and ascorbic acid (AsA) in cells of *M. aeruginosa* were affected in different ways after exposure to varying concentrations of EMA. Mohamed et al. [53] suggested that extracts of *Stratotes aloides* had a strong inhibitory effect on the growth rate, alkaline phosphatase (ALP) activity and toxicigenicity of planktonic cyanobacteria, but little effect on the growth of epiphytic cyanobacteria. The toxin production rate of epiphytic cyanobacteria was increased, which could enhance the activity of non-ribosomal peptide synthsis (NRPS) and polyketide synthases (PKSs).

**Other physiological aspects**

Allelochemicals also inhibited the growth of algae by altering their respiration, protein synthesis and gene expression [54–56]. Wink et al. [57] showed that most alkaloids have allelopathy and that they could tightly bind to the target DNA, which resulted in an increase in the cleavage temperature of DNA 5 °C and concomitantly prevented DNA translation and transcription, so that the final synthesis of proteins was affected. Moreover, action sites and intensity varies among allelochemicals and multiple targets could be selected to work together to influence cell mitosis [58]. Shao et al. [59] studied the physiological responses of *M. aeruginosa* NIES-843 under wheat bran leachate stress and showed that the expression of biological macromolecule repair genes (grpE, recA) led to no significant change, but expression of the genes for fatty acid synthesis (fabZ), antioxidant protein peroxiredoxin (prx) and the D1 protein of photosynthetic processes (psbA) were down-regulated while the gene for microcystin synthesis (mcyB) was slight up-regulated.

The mechanism by which allelopathy affects algae is via promotion of the synthesis of free radicals and reactive oxygen species (ROS). Free radicals cause harm by damaging nucleic acids, lipids and proteins [60, 61]. Usenko et al. [62] found that plant polyphenols triggered an auto-oxidation reaction that produced free radicals under alkaline conditions, and polyphenols also produced o-semiquinone free radicals in the presence of divergent or trivalent ions (e.g., Mg²⁺), which can destroy cell structure due to the strong oxidation of free radicals. ROS not only affect plant photosynthesis but also result in the disordered expression of certain genes, leading to cell aberration and cell [63, 64]. Organisms themselves produce and eliminate ROS: in other words, oxidation and antioxidation are generally in dynamic equilibrium [65]. When algal cells are subjected to various environmental stresses or noxious stimulation, the quantity of ROS and free radicals in the body will increase and its antioxidant defence systems will remove those. However, once the concentration of allelochemicals is sufficiently high to upset the balance, algal cells will suffer from oxidative damage and even die.

**POTENTIAL APPLICATIONS**

If allelochemicals were added directly to environmental water, they would have adverse impacts on aquatic organisms due to their high concentration locally [66]. Moreover, this would result in the rapid loss of various inhibitors, precluding inhibitory concentrations [67]. Therefore, the use of slow-release algicides with to inhibit algal growth may be a more effective method for controlling water blooms and reducing ecological risks [68]. Certainome natural substances, including chitosan, clay and sand, have recently been used as carriers to adsorb commonly used algaeicides such as copper sulfate [69], and herbicides [70, 71], to synthesize slow-release algicides. Bae et al. [72] utilized polylactide foam as the carrier for a naphthoquinone compound to control the harmful alga, *Stephanodiscus*. Tian et al. [73] found that the use of modified clay with *Galla chinensis* extract (0.3 g l⁻¹) produced 95% growth inhibition in *Phaeocystis globosa* and *Prorocentrum donghaiense* at 24 h, and inhibition was maintained beyond that. Ni et al. [67] prepared artemisinin anti-alkaloid-sustained-release granules using alginate-chitosan microsphere technology, and found these to have a good release property and longer inhibitory effect on *M. aeruginosa*. Huang et al. [66] showed that continuous-release beads of 5,4'-dihydroxyflavone (DHF) had better sustainable inhibitory effects on *M. aeruginosa* (>30 days) than direct DHF treatment (<10 days); the theoretical release period for DHF beads is approximately 120 days. Guo et al. [74] demonstrated that the algicide chitosan-gallate had a longer suppression time on *Microcystis flos-aquae* than that of gallic acid added directly, with both having the same inhibitory mechanism. Cheng et al. [75] used chitosan, autoclaved coal ash powder and allelochemicals containing gallic acid, azelaic acid and N-phenyl-1-naphthylamine or N-phenyl-2-naphthylamine to prepare an algicide aimed against blue-green algae. Ni et al. [76] showed that linoleic acid sustained-release microspheres reduce the production and release of algal toxins. In addition other materials, such as sand, may also be potential candidates as carriers since they can accelerate both the kinetic processes of flocculation and the sedimentation of algal flocs [77, 78], reducing the recruitment of sedimented algae and nutrient release into the surrounding water [79].

**CHALLENGES AND FUTURE PERSPECTIVE**

Allelopathic inhibition provides a novel and promising method for the control of algal blooms, and is usually considered to be environmentally friendly. Current research on the inhibitory effects of allelochemicals on algae is mostly at the laboratory stage, and there are few studies investigating the application of allelochemicals to natural water blooms. In water treatment by allelochemicals, not only should their...
algal inhibitory effects and economic benefits be considered, but also their migration, transformation and ecotoxicity in nature. Although many types of allelochemical have been extracted from plants, a relatively small number are available on account of the complicated and time-consuming process involved. Thus, it is of importance to find allelochemicals that are either abundant in plants or are artificially synthesized organic algicides with high algal inhibitory effects. Furthermore, we should pay more attention to the physiological mechanisms of allelopathy. Most current studies are conducted at the cellular level, and rarely from micro-perspectives such as gene expression and molecular and genetic mechanisms. An in-depth understanding of the mechanism of allelopathy will certainly provide a theoretical basis in the search for efficient and safe allelochemicals. In addition, future research should also be focused on the development of algicides composed of biodegradable modifiers and having multiple functions such as slow release, flocculation and sedimentation.

References


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