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EUGLENOID DERIVED ALKALOID

CROSS-REFERENCE TO RELATED APPLICATION

This present application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Ser. No. 61/100,874, which was filed on Sep. 29, 2008, the application is hereby incorporated by reference.

FIELD OF THE INVENTION

This invention relates to a purified toxin derived from *Euglena sanguinea*. More specifically the toxin, termed euglenophycin, is an alkaloid having herbicidal and cytotoxicity against plant and mammalian cells.

BACKGROUND OF INVENTION

Many factors have been documented that can contribute to mortalities observed in finfish aquaculture including disease and harmful algal blooms of cyanobacteria, in addition to more common issues with oxygen stress or nitrogen toxicosis. The source, occurrence, and epidemiology of many freshwater, estuarine, and marine toxins produced by algae are well known. For instance, divisions of photosynthetic plankton are known to produce toxins that include but are not limited to Bacillariophyceae, Pyrrophyta, Prymnesiophyta, Raphidophyta, as well as certain members of the cyanoprokaryota. Impacts from these toxins are dependant on the affected organism, as well as route, concentration, and duration of exposure.

While cyanoprokaryotic algae, diatoms, prymnesiophytes, dinoflagellates, euglenoids, and raphidophytes are long known to produce algal toxins, the identification of a toxic euglenoid is unexpected given that this species of *Euglena* that was identified by Ehrenberg in the 1830s has presented no conclusive evidence of toxin production. An exception would be a tilapia-kill event detailed in Xavier M B, et al., 1991. *Algological Studies*, 62:133-142, wherein tilapia exposed to a *Euglena sanguinea* bloom in aquaria had euglenoid cells associated with gills, resulting in distressed breathing as manifested by surface porpoising and minor tilapia fish mortality.

Euglena form protective cyst when subjected to hostile environments as a survival mechanism. This formation contributes to the difficulty in recognizing toxins produced by *euglena* as these cells encyst when water is turbulent. Other environmental factors contribute to difficult toxin identification. One scenario is that a surface scum of the euglenoid forms in calm weather during mid-morning to afternoon, resulting in high concentrations of toxin in several centimeters thickness of water. Wind events would result in dissipation of the scum through encystment, leaving a surface micro-layer containing dissolved toxins. Aquacultured fish are then fed floating feeds, resulting in concentrated exposure. These events lead to an increased difficulty in identifying a euglenophycin as the source of a toxin.

While cyanoprokaryotic algae, diatoms, prymnesiophytes, dinoflagellates, euglenoids, and raphidophytes are long known to produce algal toxins, euglenoid algae that produce toxins were isolated from aquaculture ponds, with toxin confirmation based on positive fish bioassays following exposure to the isolated clonal algal cultures. It remains an open question as to the isolation of toxin from euglenoid algae blooms at freshwater facilities.

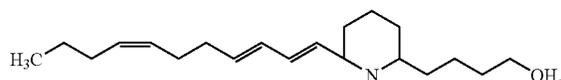
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Furthermore, while taxonomists have recognized the presence of euglenoid algae in both freshwater and marine systems, the lack of unique pigment biomarkers have prevented routine monitoring using remote sensing methodologies or HPLC pigment biomarker identification would lead to underestimation of importance of the division. Additionally, since the existence of a euglenoid toxin was only recently reported many previous fishkills caused by unidentified biological agents could be attributable to euglenoids. The apparent potency of this compound strongly suggests further assessment of occurrence in potable waters.

BRIEF SUMMARY OF THE INVENTION

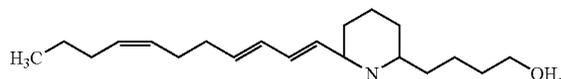
Disclosed herein is a toxin composition. The toxin composition is obtained from euglenoid algae isolates. The compound has a structure similar to alkaloids produced by fire ant venom. Advantageously, the purified toxins produced by these euglenoid isolates have activity against cancerous cell lines. Toxicity was observed in euglenoid clonal culture isolates obtained from the pond as well as a clonal, culture collection taxon. The euglenoid toxin, derived from *Euglena sanguinea* are grown in batch culture wherein the toxin is recovered and purified by techniques which are well known to those skilled in the art.

Also disclosed herein is a purified bioactive euglenophycin composition isolated from from *Euglena sanguinea* having the structure:



In one embodiment of the invention the euglenophycin is toxic against plant and mammalian cells. The compound is an alkaloid with a molecular weight of from about 288 Da to about 306 Da. In another embodiment of the invention, the euglenophycin is a herbicide and is toxic against algal cells.

Further disclosed is a method of controlling undesirable algal bloom, the method comprising contacting waterways with a herbicide composition having the formula:



In one embodiment the herbicide is present in a concentration range of about 0.3 mg per liter to about 30 mg per liter. In another embodiment, the herbicide is effective against undesirable algal bloom such as *Microcystis aeruginosa* (cyanobacteria), *Planktothrix* (cyanobacteria), *Gomphonema parvum* (diatom), *Scenedesmus dimorphus* (green algae), and *Oocystis polymorpha* (green algae).

As disclosed is a method of isolating and purifying a euglenophycin, the method comprising culturing *Euglena sanguinea* in a growth media to produce a euglenophycin therein, extracting *Euglena sanguinea* cells by separating a fraction of organic compounds from said growth media by a gradient elution of using water and acetone, and separating the gradient using a chromatography with porous silica beads. In one embodiment, the fractions are separated by a gradient of 90:10 water:acetone for 2 minutes then 20 minutes of 100%