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The algicidal activity of marine *Loktanella* sp. Gb03 on the toxic dinoflagellate *Coolia Malayensis*

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ABSTRACT

Background Toxic dinoflagellate in recent years, have become a significant problem in coastal regions worldwide because of their production of toxin. To manage and control the adverse impact of harmful algal blooms, many strategies including chemical, physical and biological methods have been developed **Objective** The aim of this study was to determine the algicidal activity of *Loktanella* sp. Gb03 against toxic dinoflagellate, as well as, the interaction between bacteria and harmful algal blooms. **Results** The algicidal bacterium, designated as *Loktanella* sp. Gb03, isolated from dinoflagellate culture (Gb03 sequences were deposited in NCBI GenBank with the accession numbers; *Loktanella* sp. strain UKMGb03A (KU199217)), was assumed to produce secondary metabolites. When 10% culture filtrate of this strain was applied to *C.malayensis* cultures, over 99% of *C. malayensis* cells were destroyed after 24 hours. Gb03 strain showed significant algicidal activities against *C.malayensis*, and wide algicidal range against various harmful algal bloom (HABs) species. **Conclusion:** This study is the first study report of the algicidal activity of the *Loktanella* sp. against toxic dinoflagellate *C.malayensis*. And also to get more information about bacteria-algal interaction. Taken together, our results suggest that Gb03 could be a candidate for controlling HABs.

KEY WORDS

algicidal bacteria, *Loktanella* sp., harmful alga blooms, dinoflagellate, *Coolia Malayensis.*

INTRODUCTION

Harmful algal bloom (HAB) events contaminate coastal life. It has seriously damage the aquaculture industries [1] as well as environmental and human health [2, 3]. Each year, around two thousand cases of human poisoning due to algal toxins were reported [4]. Coastal HAB events have been estimated to result in economic impacts in the United States of at least $82 million per year. These impacts stress the importance of understanding HABs and developing tools to mitigate their impacts and ultimately to control or prevent the algal blooms.

Several methods had been developed to manage the HABs that include Physical/chemical/biology method. Method such as Yellow loess [5] and clay [6] had been used to manage harmful algal blooms (HABs). However, both methods caused a secondary effect on the bottom-dwelling organisms [7]. Chemical agents such as triosyn, copper sulfate, and hydrogen peroxide were used to control HABs for a short term period [8, 9]. They also had a significant side effect as well [3]. Another strategy involved using biological method to control HABs. Several studies had demonstrated the application of bacteria [10-12], viruses[13], and Protozoa[14] to control HABs. There are several strains of bacteria have ability to kill or inhibit the growth of HABs species [3, 15].

Marine bacteria consider as source of natural algicides which can play an important role by controlling the HABs. In this study, we demonstrate the algacidal activity of a bacterium isolated from dinoflagellate culture against the toxic dinoflagellate *C.malayensis.* To the best of our knowledge, there are many studies investigating marine algicidal bacteria against toxic dinoflagellates in Malaysia water, but this is the first study on *Loktanella* sp. Gb03. We hypothesized the isolated bacteria from dinoflagellate culture has very strong algicidal activity against wide range of dinoflagellates species. Therefore, this study was aimed to investigate the algicidal activity of *Loktanella* sp, Gb03 followed by its mode of action and stability of algicidal compounds.

MATERIAL AND METHODS

Culture condition of alga and algicidal bacteria:

Dinoflagellates *Coolia Malaynesis, Alexandrium tropicale, A. leei, A. affine, A. tamiyavanichi, A. tamarense, Gambierdiscus belizeanus* and *Ostreopsis* (kindly supplied by Professor Dr. GiresUsup, School of Environmental and Natural Resources Sciences, Faculty of Science and Technology, University Kebangsaan Malaysia,43600 (UKM) Bangi, Malaysia) was cultivated in ES-DK medium[16] prepared using natural sea water[17, 18] under a light intensity of 140 µmol $m^{-2}s^{-1}$ and 12:12 hours light:dark photoperiod [19, 20].

Loktanella sp. Gb03 was isolated from dinoflagellate culture (*Gambierdiscus Belizeanus*) in laboratory by serial dilution technique on nutrient agar. Strain Gb03 was maintained on nutrient agar at 4°C for short-term preservation and in a glycerol suspension (20%, w/v in distilled water) at -80°C for long-term preservation.

Algicidal effect of bacterium Gb03 strain:

Relationship between algicidal activity and growth curve:

The algicidal activity of Gb-03 strain and its growth curve were investigated every 3 hours for 39 hours. The bacterium Gb-03 strain was first inoculated in marine broth and incubated at optimal culture condition at 30°C, pH 7, 1% NaCl and 200 rpm shaking. The cell growth was determined by using spectrophotometer (540nm). Briefly, the optical density (OD) was estimated every 3 hours for 39 hours. To determine the algacidal activity, a 100 µL of filtrated culture supernatant were taken to test the activity at different growth stages. One mL of *C.malayensis (*3x10^3 cells/mL) was placed in each well of 24-well plate. After that, a 100µL of bacteria filtrated culture supernatant at every stage was added into each well (i:e every three hours). The plate was kept at algal culture condition for 24 hours and the cells were observed by using counting chamber (Sedgwick-Rafter Cells).

Algicidal activity effect at different volume of filtrated culture supernatant of Gb03 on C.malayensis marine:

Culture of Gb03 strain in marine broth for 36 hours at 30°C, were centrifuged at 15000xg for 15 minutes, and followed by filtering the supernatant through 0.2µm Millipore membranes. Each five tubes contain 1mL of sterilized nutrient broth at different volume (10, 50, 100, 500 or 1000 µL) of filtrated supernatant of *Loktanella* sp. Gb03 were added to the tubes. And by using 24-well plate, the algicidal activity of strain Gb03 was tested against *C.malayensis* (1.0 *10^3 cells/mL). Each well contain 1 mL of *C.malayensis* culture, to which 10% (V/V) from the prepared solution were added. The plates were monitored at 100x magnification. The plates were inspected after 24 hours incubation time in alga condition (light intensity of 140 µmol $m^{-2}s^{-1}$ and 12:12 hours light:dark photoperiod).

Algicidal activity of bacteria Gb03 strain against other species of dinoflagellate:

The algicidal activity of Gb03 strain was investigated against other harmful algae blooms species. The Gb03 strain culture filtrate was prepared as described above. In this experiment, the algae species that were used were *Alxandrium tropicale, A. leei, A. affine, A. tamiyavanichi, A. tamarense, Gambierdiscus belizeanus,* and *Ostreopsis.* Ten percent of filtrated culture supernatant of Gb03 was added to each species at the stationary growth phase (1.0 × 10^3 cells/ mL). The test flask kept for 24 hours in algal culture condition.

The stability of algicidal activity:

The preparation of filtrated supernatant from Gb03 strain culture was described above. The filtrated supernatant was tested against different temperature and pH to determine the stability of algacidal activity. To test the most ability of algacidal compound, the filtrated supernatant was incubated in a water bath at 4, 20, 40, 50, 70 and 100°C for two hours. On the other hand, the stability of the filtrate in different pH was tested by adding different amount of acetic acid or NaOH. pH from 3 to 10. The incubation was maintained (at room temperature) for two hours, followed by returning the pH to 7 before testing against dinoflagellate. (10% v, v) of treated filtrate were added to the *C.malayensis* culture (1.0 × 10^3 cell/mL), to determine the algicidal activity. Marine broth (10% v, v) was added as a control.

Statistical analysis:

Calculation of algicidal activity of Gb-03 strain was done by using the following equation [21] : $Algicidal\ activity\ (\%) = (1 - Tt/Ct) \times 100$. Tt (treatment) and Ct (control) are the cell concentrations of *C.malayensis* marina with culture supernatants of Gb03 strain, and sterile marine broth, respectively, after inoculation time (t). Exact volumes of the culture supernatants was inoculated to the cultures of *C.malayensis* marina in the treatment. Equal volumes of Es-Dk medium [16] and sterilized marine broth were added to the *C.malayensis* marine culture as a two positive controls instead of filtrated bacterial culture supernatant. Triplicate test were carried out for all the test over mentioned. The algicidal activity was analyzed using one-way analysis of variance and compared using Duncan's test at a significance level of $P<0.05$ using SPSS v10.1 (SPSS Inc., USA).

Results:

Relationship of algicidal activity and bacterial growth:

The algicidal activity of Gb03 strain was increasing proportionally to the optical density (figure.1). Therefore, the effect of algicidal activity was dependent on the bacterial growth. The strongest algicidal activity was shown during the early stationary phase of Gb03 strain culture.

Algicidal activity effect at different volume of filtrated culture supernatant of Gb03 on C.malayensis:

Five different volume of filtrated culture supernatant were tested in this experiment. High concentration of culture supernatant had been shown strong activity on *C.malayensis* culture, the algicidal activity increase by increasing the volume of culture supernatant of Gb03 strain. Adding 10% of the first and second prepared diluted (10 and 50 µL) of culture supernatant had shown very weak activity, but when third, fourth, and fifth prepared diluted (100, 500 and 1000) of filtrated culture had been shown strong algicidal activity, and it could kill 100% of *C.malayensis* after 24 hours incubation time at alga condition culture (figure.2). The same amount of marine broth or Es-dk medium was added as a two positive control.

Algicidal activity of Gb03 strain culture supernatant on other harmful algae blooms:

The strain Gb03 filtrated culture supernatant also showed effect on another HABs (Figure.3). Filtrated culture supernatant of Gb03 strain shown strong algicidal activity on some of dinoflagellate been tested. The results of the seven dinoflagellate been tested as follows: *A.tropicale* 70%, *A.leei* 50%, *A.affine* 95%, *A.tamiyavanichi* 70%, *A.tamarense* 90%, *Gambierdiscus belizeanus* 90%, and *Ostreopsis* 60%.

Stability of algicidal activity:

The culture filtrate also showed algicidal activity after being treated with different rang of temperature (4, 20, 40, 50, 70 and 100°C) for two hours. That means the algicidal compounds are heat-tolerant (Figure.4). Strain Gb-03 also shown algicidal activity in different range of pH (Figure.5).

Discussion:

There are many marine bacteria reported to have algicidal activity on various species of harmful algal blooms and red tide phytoplankton[12, 22]. Almost 45% of the marine bacteria with algicidal activity have been reported belong to γ-proteobacteria[23, 24]. And about 50% of algicidal strain belong to the CFB group, and the remaining strains represent the Gram positive genera Micrococcus, Bacillus, and Planomicrobium [24, 25]. This bacterial isolate closely related to *Loktanella sp*. was unusual, because most algicidal bacteria belong to either Cytophaga–Flavobacterium–BacteroidesCFB group or the genus Pseudoalteromonas [22, 26]. Research on the relationship and interactions between marine bacteria and phytoplankton is urgently needed because it has been found to play a major role in HABs controlling [27].

Algicidal from marine bacteria play an important role by killing or inhibiting the growth of harmful algal bloom in coastal sea water where red tides occur frequently [11]. Marine bacteria are the main biological controllers in the dramatic termination of harmful algal blooms in coastal seawaters [28]. The identification of the algicidal compound is difficult because of the several species of algicidal bacteria [26]. Several compound reported as algicidal compounds also have antibiotic-like substances [29], biosurfactants [30, 31], proteases [32], peptides [33], gargimicin A [33], or Prodigiocin-like pigments [34].

Related to the results of the stability of algicidal activity experiment, we came to know that is the algicidal compounds of Gb03 strain is not enzymatic, since the filtrate after incubated in water bath for two hours at 100°C, showed almost the same activity. There is some algicidal compounds been tolerate the autoclaving, so it can't be enzymatic event because of the high temperature enzymes [26]. We expected Gb-03 strain used one of the aforementioned compounds as algicidal compound to kill the *C.malayensis* marine.

Therefore, our results showed that the algicidal bacterium *Loktanella* sp. Gb03 can function as a red-tide controller. Recent work has been focusing on algicidal bacteria as controllers of water blooms.

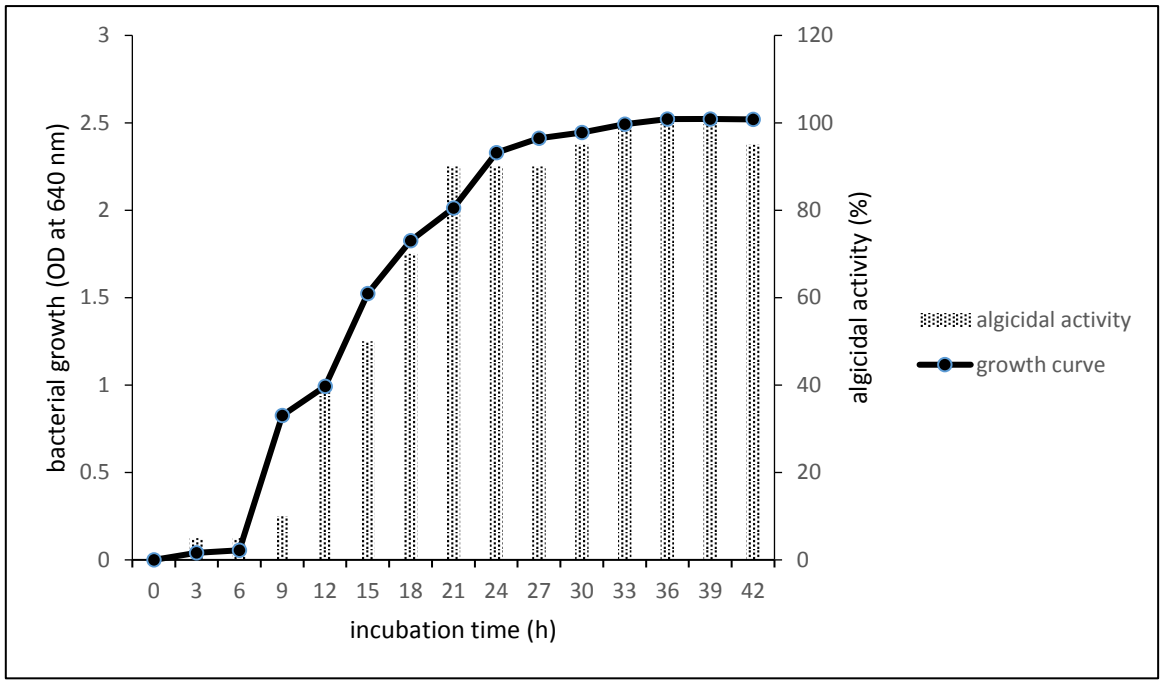

Fig. 1: Growth curve of *Loktanella* sp. Gb03 at optimal culture conditions (i.e., 30°C, pH 7, 1% NaCl, 200 rpm) and algicidal activity by the culture supernatant of each growth phase of *Loktanella sp*. Gb03 against *C.malayensis* marine.

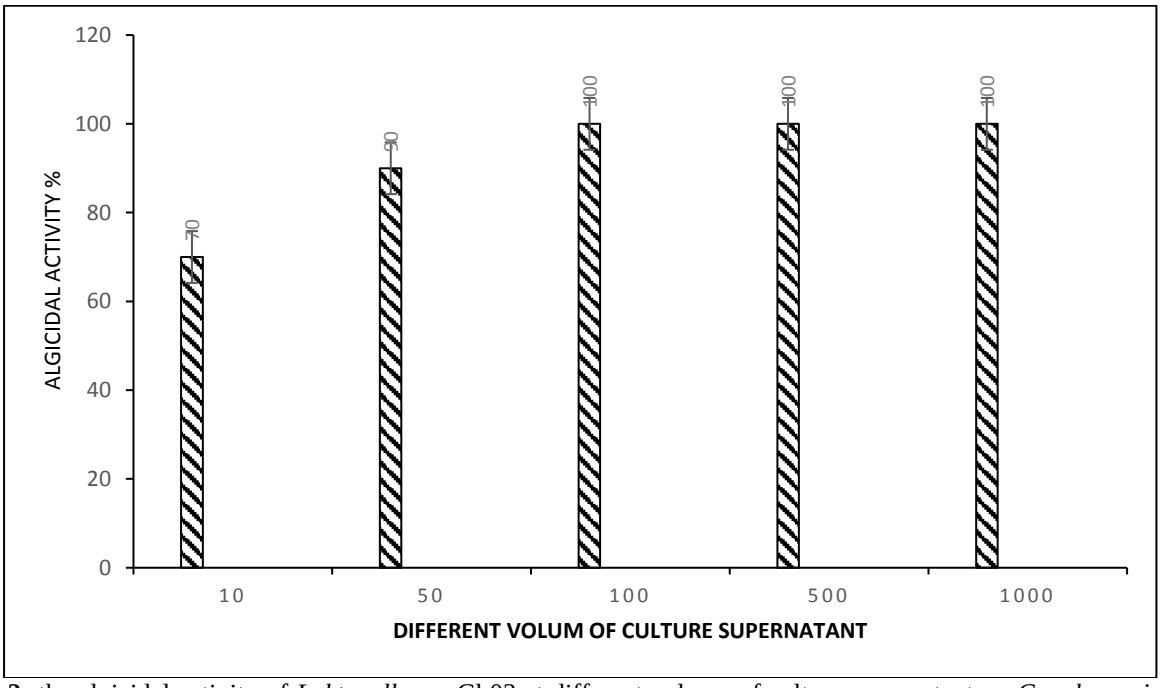

Fig. 2: the algicidal activity of *Loktanella* sp. Gb03 at different volume of culture supernatant on *C.malaynesis* marine.

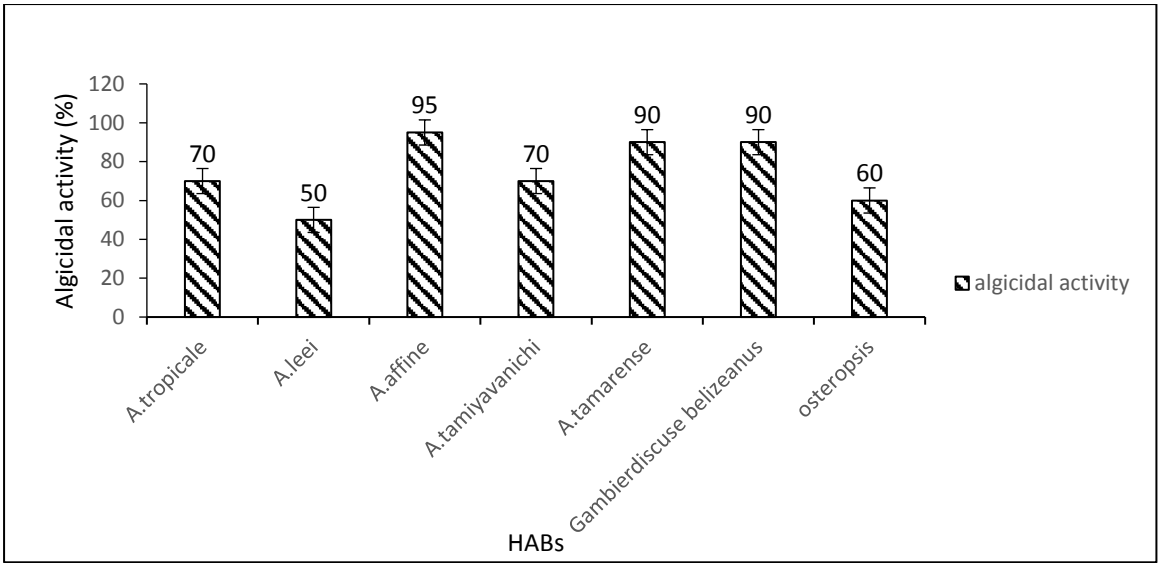

Fig. 3: The algicidal activity of *Loktanella* sp. Gb03 against other dinoflagellate species. The 10% culture supernatant was added to each algal culture at the mid-exponential growth phase. Control=each algal culture with sterile marine broth.

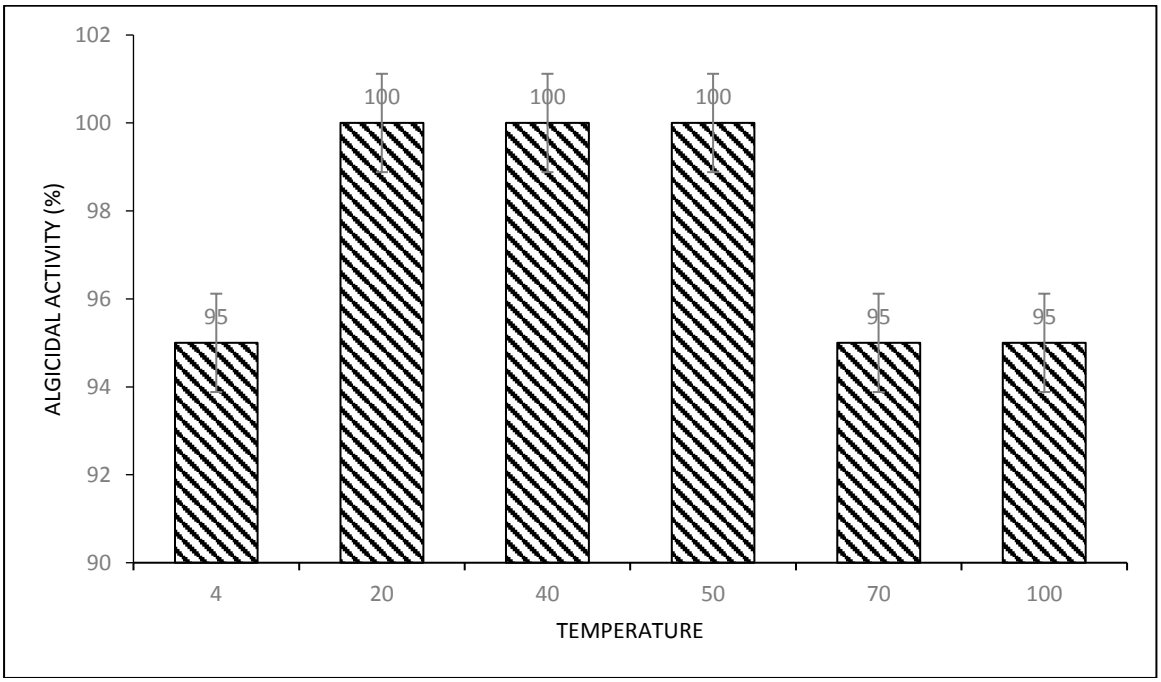

Fig. 4: Stability of culture supernatant of *Loktanella* sp. Gb03 at different rang of temperature. The culture filtrate was incubated at temperature (4, 20, 40, 50, 70, and 100°C) for two hours.

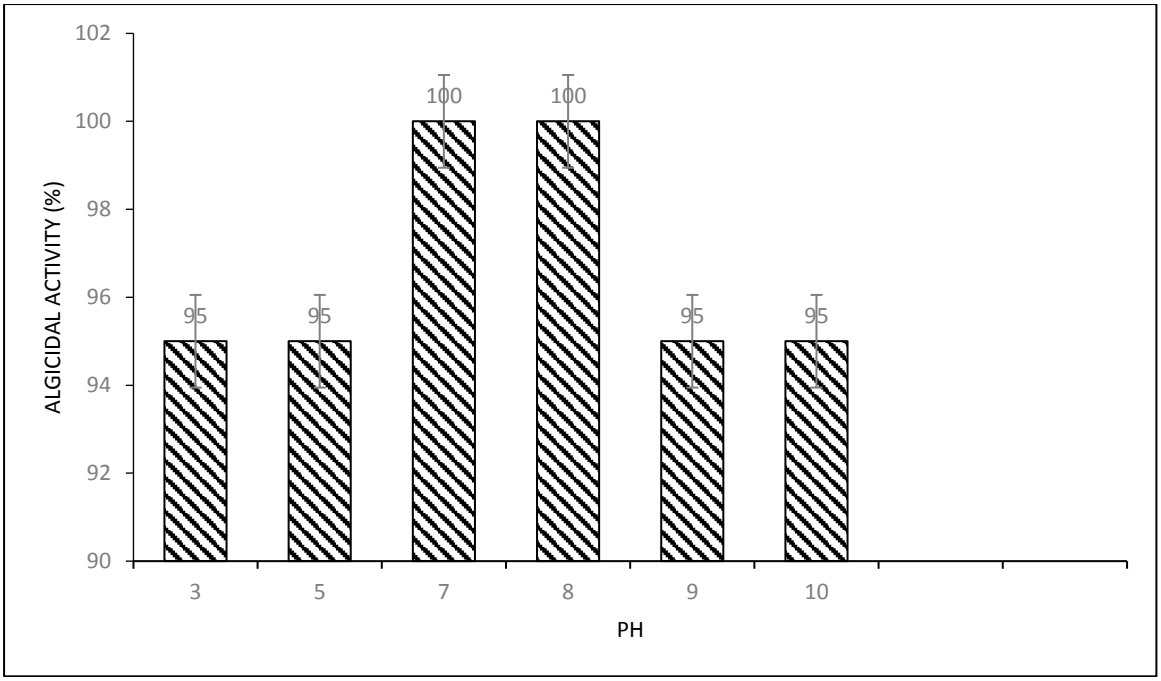

Fig. 5: pH stability of culture filtrates of *Loktanella* sp. Gb03

Conclusion:

However, conclusion of the present investigation *Loktanella* sp. Gb03 contain algicidal activity can play an important roles for controlling the Harmful Algae Blooms (HABs). Related to the results of relationship of algicidal activity and bacterial growth, suggested that the algicidal activity increased by increasing the optical density of the bacterial broth culture. As well as, more volume of bacterial supernatant showed more algicidal activity. However, the *Loktanella* sp. Gb03 supernatant has algicidal activity on other toxic dinoflagelate as well. The *Loktanella* sp. Gb03 supernatant was stable at different range temperature and different range of pH. However, the further studies will include extraction and characterization of bioactive compounds from the algicidal bacteria *Loktanella* sp. Gb03.

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