EXTRACTION AND CHARACTERIZATION OF ALGICIDAL COMPOUNDS FROM ALGICIDAL BACTERIA LOKTANELLA SP. Gb03 AND ITS ACTIVITY AGAINST TOXIC DINOFLAGELLATE COOLIAMALAYENSIS

Article · January 2016

CITATIONS
0

READS
76

5 authors, including:

Anmar Hameed Bloh
Al-Rafidian University college
15 PUBLICATIONS **23** CITATIONS
SEE PROFILE

Gires Usup
Universiti Kebangsaan Malaysia
159 PUBLICATIONS **2,097** CITATIONS
SEE PROFILE

Asmat Ahmad
Universiti Kebangsaan Malaysia
108 PUBLICATIONS **1,044** CITATIONS
SEE PROFILE

Ali Abd Sharad
University of Anbar
30 PUBLICATIONS **46** CITATIONS
SEE PROFILE

Scientific Reviews
&
Chemical Communications

Sci. Revs. Chem. Commun.: 6(4), 2016, 84-90
ISSN 2277-2669

EXTRACTION AND CHARACTERIZATION OF ALGICIDAL COMPOUNDS FROM ALGICIDAL BACTERIA *LOKTANELLA* SP. Gb03 AND ITS ACTIVITY AGAINST TOXIC DINOFLAGELLATE COOLIAMALAYENSIS

ANMAR HAMEED BLOH[a], ALI ABDSHARAD[b], GIRES USUP[c] and ASMAT AHMAD[*]

School of Biosciences and Biotechnology, Faculty of Science and Technology,
University Kebangsaan Malaysia, 43600 (UKM) BANGI, MALAYSIA
[a]School of Biosciences and Biotechnology, Faculty of Science and Technology,
University Kebangsaan Malaysia, 43600 (UKM) BANGI, MALAYSIA
[b]School of Biosciences and Biotechnology, Faculty of Science and Technology,
University Kebangsaan Malaysia, 43600 (UKM) BANGI, MALAYSIA
[c]School of Environmental and Natural Resources Sciences, Faculty of Science and Technology,
University Kebangsaan Malaysia,43600 (UKM) BANGI, MALAYSIA

(*Received : 31.10.2016; Revised : 09.11.2016; Accepted : 12.11.2016*)

ABSTRACT

Harmful algal blooms have caused huge damage to the marine ecosystem and the coastal economy all over the world. To identify a capable bio-agent of controlling dinoflagellate blooms, a bacterial strain Gb03 was isolated, which showed strong algicidal activity against toxic dinoflagellate *Cooliamalayensis*. The aim of this study is to extract and identify the active compounds of algicidal bacteria. The algicidal bacterium, designated *Loktanella*sp. Gb03 (Gram-stain-negative, non-motile, non-spore-forming, short rod-shaped, aerobic bacterium, was isolated from dinoflagellate culture in laboratory), was assumed to produce secondary metabolites[1]. The results of this study also suggested the algicidal activity of strain Gb03 occurred by production of algicidal compounds as extracellular. Subsequently, the algicidal substances from strain Gb03 culture were isolated and purified by Column chromatography and thin layer chromatography. Investigation of the algicidal compounds revealed that there were more than onealgicidal compounds produced by strain Gb03. These results indicated that strain Gb03 has great potential for use in the control of outbreaks of toxic dinoflagellate.

Key words: Algicidal bacteria, Dinoflagellate, Loktanella, Cooliamalaynesis, Harmful algal blooms and Andalgicidal compounds.

INTRODUCTION

Harmful algal blooms (HABs) in recent years, have become a significant problem in coastal regions worldwide because of their production of toxin, sheer biomass and physical shape[2-4]. To manage and control

Available online at www.sadgurupublications.com
[*]Author for correspondence; E-mail: anmarhameed@yahoo.com, aliabd197311@yahoo.com.sg, asmat@ukm.edu.my, gires@ukm.my; Phone: +60123567341, +60127206261, 0193883949, 0126365400; Fax: 89252698

the adverse impact of harmful algal blooms, many strategies including chemical, physical and biological methods have been developed[5].

Although some of these strategies are not suitable for controlling HABs, for example physical methods can control HABs within a short period after application. Also Chemical methods as another example are easy to operate and can control the algal blooms, but they also can cause secondary pollution. However, biological methods such as algicidal bacteria play important roles to control HABs[6,7].

In recent years, many strains of algicidal bacteria isolated from natural ecosystem, such as *Vibrio sp*.,[8] *Actinomyces sp*.,[7] *Flavobacterium sp*.,[9] *Pseudoalteromonas sp*.[10] and *Streptomycete sp*.[11]

However, all algicidal bacteria inhibit the growth of algae by attacking the cells either directly by cell-to-cell contact or indirectly by the release of algicdal substances[12,13], such as antibiotics[14], proteases[15], bio-surfactants[16,17], peptides[18] and amino acids[19]. Algicidal bacteria bioactive compounds have been isolated and characterized around the world. The screening of algicidal compounds consideredas important tools to investigate new bioactive compounds, which consider as important fighter against harmful algae.

In previous study[1], we investigated the isolation and identification of an effective algicidal bacterium against *C. malaynesis*, the algicidal activity of the *Loktanella* sp. Gb03 bacterial strain against C. *malaynesis*, the algicidal range of the isolated strain against other toxic algae species, and now will study the chemical compounds extracted from the bacterial culture of strain Gb03 and the ability to inhibit the growth of C. *malaynesis*.

EXPERIMENTAL

Material and methods

Culture condition of alga and algicidal bacteria

Dinoflagellates *Coolia Malaynesis*, (kindly supplied by Professor Gires Usup, 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[20] prepared with natural sea water[21] under a light intensity of 140 μmol $m^{-2}s^{-1}$ and 12:12 hrs light: dark photoperiod.

And *Loktanella*sp. Gb03 was isolated from dioflagellate culture (*Gambierdiscus Belizeanus*) in laboratory by serial dilution technique on nutrient agar. Strain Gb-03 was maintained on nutrient agar at 4°C for short-term preservation and as a glycerol suspension (20%, w/v in distilled water) at -80°C for long-term preservation[22].

Preparation of crude Extract from *Loktanellasp*. Gb03 and its activity on C. *malayensis*

In this experiment, Culture of Gb03 strain in marine broth for 36 hrs at 30°C, were centrifuged at 15000 xg for 15 min, and followed by filtering the supernatant through 0.2 μm Millipore membranes, we extracted from filtrated supernatant by using organic solvents Ethyl acetate at ratio of 1:1 (v:v). The supernatant was mix with solvent (overnight) in separation flask. The solvent was evaporated until minimum amount, and was drying for three days[23]. The dried extract was dissolved in DMSO for further study. From ethyl acetate extract, 10 μL was added into 1 mL of dinoflagellate *C. malayensis* culture in 24-well micro plates. The plate were incubated at the algae condition growth (light intensity of 140 μmol $m^{-2}s^{-1}$ and 12:12 hrs light: dark photoperiod) for 24 hrs and the cells were observed under microscope by using counting chamber (Sedgwick-Rafter Cells (grid)).

Column chromatography

The extract dissolved in ethyl acetate and subjected to column chromatography (commercial silica gel, Merck, 230-400 mesh; 1 × 50 cm). Separation depends on the color of the fraction. Each fraction of the effluent of column chromatography was collected and evaporated; after which, it was dissolved in 0.2 mL of DMSO. The algicidal effects of each fraction were evaluated based on the dinoflagellate assay by using 24-well plate over mentioned.

Thin layer chromatography

Thin layer chromatography (TLC) was employed to analyze the compounds present in the fractions after column chromatography. The fractions were applied to a TLC plate about 1.3 cm from the edge (spotting line), using 20 µL capillary tubes (microcaps disposable pipettes, Drummond Scientific Company). All TLC separations were performed at room temperature, i.e. 18-23°C. After sample application 3 µL of fractions, the plates were placed vertically into a solvent vapor saturated TLC chamber. After the mobile phase (Ethyl Acetate) had moved about 80% from the spotting line, the plate was removed from the developing chamber and dried in a fume hood the plate was visualized at UV–254 nm, spots were circled and the plate was sprayed with 10% ethanolicphosphomolybdic acid reagent (purchased from Sigma Aldrich). The TLC plate was dried 5-10 min under a fume hood and heated at 100°C for 3-5 min under observation[24].

Activity of fractions on toxic dinoflagellate *Coolia malaynesis*

Ten microliter from each fraction (1 mg/mL), was added into 1mL of dinoflagellate (*Coolia Malaynesis)* culture in 24-well micro plate. The plate were incubated at the algae condition growth (light intensity of 140 µmol $m^{-2}s^{-1}$ and 12:12 hr light:dark photoperiod) for 24 hr. Algicidal activity was determined using counting chamber (Sedgwick-Rafter Cells (grid)). DMSO were used as a control.

RESULTS AND DISCUSSION

Preparation of extract and its activity on toxic *dinoflagellate C. malaynesis*

Out of almost 60 L of the cultured filtrated supernatant, we extracted around 2.9 g, used ethyl acetate as solvent. One gram of the dried extract was dissolved in 2.5 mL, around 10 µL of this dissolved extract showed 100% activity against *C. malaynesis.*

Column chromatography

A total of 48 fractions were collected from Column chromatography (Fig. 1). The TLC profile of the fractions collected showed some similar spots. Tubes of fractions were pooles based on TLC similarities. Five combined fractions were obtained (Gbf1 to Gbf5) and the mass of each fraction was recorded (Table 1).

Table 1: Mass of fractions (Gbf1 to Gbf5)

Fraction	Mass (mg)
Gbf1	490.4
Gbf2	460.9
Gbf3	420.7
Gbf4	370.4
Gbf5	220.5

Fig. 1: Fractionation by column chromatography

Algicidal activity of column chromatography fractions

Among the tested fractions (Gbf1- Gbf5), fraction Gbf1 showed highest activity against toxic dinoflagellate *C.malaynesis* after six hours incubation time. The results of all the five fractions were as follow: 100%, 30%, 60, 70%, 70% and 60%, respectively (Fig. 2).

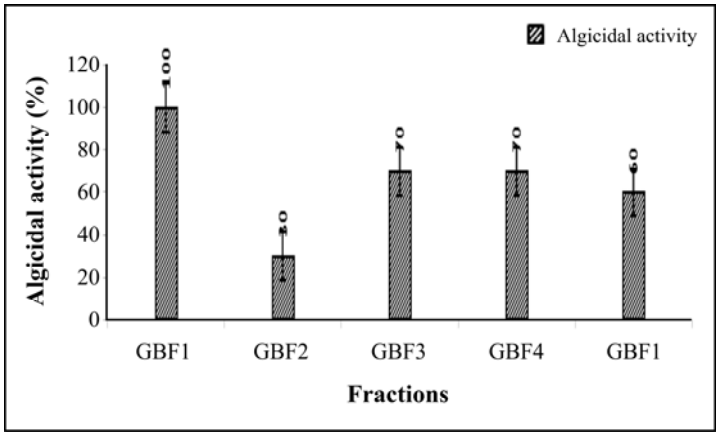

Fig. 2: Algicidal activity of column chromatography fractionson *C.malaynesis*, while using nutrient broth medium extract by ethyl acetate as a controland DMSO as a second control

The search for algicidal activity from bacterial source has received much attention and efforts have been put in to identify compounds that can act as suitable algicidal agent against HABs to replace synthetic ones. Harmful algal blooms (HABs) have been a serious problem for public health and fisheries industries in recent years. Algicidal bacteria releasing compound which is called algicidal compounds, with less toxic and more solution to control the growth of harmful microorganism[8,25,26]. Algicidal compounds have significant efforts against human pathogens such as toxic algae. Many studies conducted with the bacteria extracts, screening algicidal activity and for investigate of new algicidal compounds. Based on previous studies on the algicidal compound and algicidal activity, the algicidal compounds can be alkaloids[27], proteins[28], peptides[29], antibiotics[30], amino acids[19], pigments[31] and biosurfactants[17]. In this investigation, Ethyl acetate extract of *Loktanella* sp. Gb-03, was evaluated for exploration of their algicidal activity against toxic dinoflagellates which regarded as harmful for human microorganisms. In this study the algicidal effects of bacterial (*Loktanella* sp. Gb-03) extract against *C.malaynesis* dinoflagellate were recorded. It was concluded that the algicidal effects of Loktanella sp. Gb03 has strong activity and power to kill dinoflagellate

C.malaynesis after 24 hrs incubation time. The characterization of the compounds is urgently needed. Harmful blooms frequently occurred around the world since its first recorded observation around 1923[32]. These blooms have negative effects on the marine ecosystem, and that influenced human activities like fisheries and coastal tourism[33,34]. However, it is urgent to study the interactions between HABs and bacteria, and develop harmful microbial control methods. In this study, we extract compounds from *Loktanella*sp. Gb-03 by using Ethyl Acetate, the organic extracts using Ethyl acetate as solvent provided more powerful algicidal activity as compared to direct supernatant.

CONCLUSION

However, conclusion of the present investigation *Loktanella* sp. Gb-03 contain algicidal activity and multialgicidal compounds which can play an important role for controlling the Harmful Algae Blooms (HABs). Related to the results of Column chromatography and Activity of fractions on toxic dinoflagellate, we suggested all the fractions has ability to control Harmful Algae Blooms (HABs).

ACKNOWLEDGMENT

The authors thank Dr. Fareed Sairifor his contributions to this work. This work was supported by grants 04-01-SF14 and UKM GUP BTK 07-75-198.

REFERENCES

1. C.-Y. Ahn, S.-H. Joung, J.-W. Jeon, H.-S. Kim, B.-D. Yoon and H.-M. Oh, Selective Control of Cyanobacteria by Surfactin-Containing Culture Broth of Bacillus Subtilis C1, Biotechnol. Lett., **25(14)**, 1137-1142 (2003).
2. Donald M. Anderson, Turning Back the Harmful Red Tide, Nature, **388(6642)**, 513-514 (1997).
3. F. Azam, Microbial Control of Oceanic Carbon Flux: The Plot Thickens, Science, **280(5364)**, 694-696 (1998).
4. E. Banin, S. K. Khare, F. Naider and E. Rosenberg, Proline-Rich Peptide from the Coral Pathogenvibrio Shiloi That Inhibits Photosynthesis of Zooxanthellae, Appl. Environ. Microbiol., **67(4)**, 1536-1541 (2001).
5. A. H. Bloh, G. Usup and A. Ahmad, Loktanella Spp. Gb03 as an Algicidal Bacterium, Isolated from the Culture of Dinoflagellate Gambierdiscus Belizeanus, Veterinary World, **9(2)**, 142 (2016).
6. W. M. Chen, F. S. Sheu and S. Y. Sheu, Novel L-Amino Acid Oxidase with Algicidal Activity against Toxic Cyanobacterium Microcystis Aeruginosa Synthesized by a Bacterium Aquimarina Sp., Enzyme and Microbial Technol., **49(4)**, 372-379 (2011).
7. J. Y. Cho, Algicidal Activity of Marine Alteromonas Sp. Kns-16 and Isolation of Active Compounds Bioscience, Biotechnol. Biochem., **76(8)**, 1452-1458 (2012).
8. B. E. Ezema, F. U. Eze and C. C. Ezeofor, Phytochemical and Antibacterial Studies of Eastern Nigerian Mistletoe (Loranthus Micranthus) Parasitic on Pentacletra Macrophylla and Parkia Biglobosa, **7(5),** 123-124(2010).
9. S. Fan, M. Fu, Z. Wang, X. Zhang, W. Song, Y. Li, G. Liu et al., Temporal Variation of Green Macroalgal Assemblage on Porphyra Aquaculture Rafts in the Subei Shoal, China, Estuarine, Coastal and Shelf Science, **163**, 23-28 (2015).

10. R. Gao, B. Cao, Y. Hu, Z. Feng, D. Wang, W. Hu, J. Chen et al., Human Infection with a Novel Avian-Origin Influenza a (H7n9) Virus, New England J. Medicine, **368(20)**, 1888-1897 (2013).

11. R. R. L. Guillard, Culture of Phytoplankton for Feeding Marine Invertebrates, In Culture of Marine Invertebrate Animals, Springer (1975) pp. 29-60.

12. N. Imamura, I. Motoike, M. Noda, K. Adachi, A. Konno and H. Fukami, Argimicin a, a Novel Anti-Cyanobacterial Compound Produced by an Algae-Lysing Bacterium, The J. Antibiotics, **53(11)**, 1317-1319 (2000).

13. S.Kodani, A. Imoto, A. Mitsutani and M. Murakami, Isolation and Identification of the Antialgal Compound, Harmane (1-Methyl-B-Carboline), Produced by the Algicidal Bacterium, Pseudomonas Sp. K44-1, J. Appl. Phycol., **14(2)**, 109-114 (2002).

14. J. P. Kokinos and D. M. Anderson, Morphological Development of Resting Cysts in Cultures of the Marine Dinoflagellate Lingulodinium Polyedrum (= L. Machaerophorum), Palynol., **19(1)**, 143-166 (1995).

15. S.-O. Lee, J. Kato, K. Nakashima, A. Kuroda, T. Ikeda, N. Takiguchi and H. Ohtake, Cloning and Characterization of Extracellular Metal Protease Gene of the Algicidal Marine Bacterium Pseudoalteromonas Sp. Strain A28, Biosci. Biotechnol. Biochem., **66(6)**, 1366-1369 (2002).

16. C. Lovejoy, J. P. Bowman and G. M. Hallegraeff, Algicidal Effects of a Novel Marinepseudoalteromonas Isolate (Class Proteobacteria, Gamma Subdivision) on Harmful Algal Bloom Species of the Generachattonella, Gymnodinium, Andheterosigma, Appl. Environ. Microbiol., **64(8)**, 2806-2813 (1998).

17. J. Luo, Y. Wang, S. Tang, J. Liang, W. Lin and L. Luo, Isolation and Identification of Algicidal Compound from Streptomyces and Algicidal Mechanism to Microcystis Aeruginosa, PloS one, **8(10)**, e76444 (2013).

18. X. Mayali and G. J. Doucette, Microbial Community Interactions and Population Dynamics of an Algicidal Bacterium Active against Karenia Brevis (Dinophyceae), Harmful Algae, **1(3)**, 277-293 (2002).

19. T. Nakashima, D. Kim, Y. Miyazaki, K. Yamaguchi, S. Takeshita and T. Oda, Mode of Action of an Antialgal Agent Produced by a Marine Gammaproteobacterium against Chattonella Marina, Aquatic Microbial Ecology, **45(3)**, 255-262 (2006).

20. T. Nakashima, Y. Miyazaki, Y. Matsuyama, W. Muraoka, K. Yamaguchi and T. Oda, Producing Mechanism of an Algicidal Compound against Red Tide Phytoplankton in a Marine Bacterium Γ-Proteobacterium, Appl. Microbiol. Biotechnol., **73(3)**, 684-690 (2006).

21. P. D. Nichols, J. H. Skerratt, A. Davidson, H. Burton and T. A. Mcmeekin, Lipids of Cultured Phaeocystis Pouchetii: Signatures for Food-Web, Biogeochemical and Environmental Studies in Antarctica and the Southern Ocean, Phytochem., **30(10)**, 3209-3214 (1991).

22. H. W. Paerl and J. Huisman, Blooms Like It Hot, Science-New York Then Washington, **320(5872)**, 57 (2008).

23. J. M. Salman and E. Abdul-Adel, Bioresponsce of Microalgae Oscillatoria Limnetica to Organophosphorous Pesticide Glyphosate, **53(1)**, 43-56 (2012).

24. J. M. Salman, E. Abdul-Adel and A. F. AlKaim, Effect of Pesticide Glyphosate on Some Biochemical Features in Cyanophyta Algae Oscillatoria Limnetica, **47(4)**, 39-46 (2009).

25. A. Saputra, Sri Andayani and Happy Nursyam, Total Quantity of Phenol and Isolation Methanol Tannin Extract of Red Betel Leaf (Piper Crocatum), **63(5)**, 195-199 (2013).

26. V. Schoemann, S. Becquevort, J. Stefels, V. Rousseau and C. Lancelot, Phaeocystis Blooms in the Global Ocean and Their Controlling Mechanisms: A Review, J. Sea Res., **53(1)**, 43-66 (2005).

27. T. Somdee, N. Sumalai and A. Somdee, A Novel Actinomycete Streptomyces Aurantiogriseus with Algicidal Activity against the Toxic Cyanobacterium Microcystis Aeruginosa, J. Appl. Phycol., **25(5)**, 1587-1594 (2013).

28. D. Suryanto and E. M. N. Yeldi, Antifungal Activity of Endophyte Bacterial Isolates from Torch Ginger (Etlingera Elicitor (Jack.) Rm Smith)) Root to Some Pathogenic Fungal Isolates, **77(17)**, 77-82 (2007).

29. B. X. Wang, Y. Y. Zhou, S. J. Bai, J. Q. Su, Y. Tian, T. L. Zheng and X. R. Yang, A Novel Marine Bacterium Algicidal to the Toxic Dinoflagellate Alexandrium Tamarense, Lett. Appl. Microbiol., **51(5)**, 552-557 (2010).

30. H. Wang, W. Zhang, L. Chen, J. Wang and T. Liu, The Contamination and Control of Biological Pollutants in Mass Cultivation of Microalgae, Bioresource Technol., **128**, 745-50 (2013).

31. X. Wang, L. Gong, S. Liang, X. Han, C. Zhu and Y. Li, Algicidal Activity of Rhamnolipid Biosurfactants Produced by Pseudomonas Aeruginosa, Harmful Agae, **4(2)**, 433-443 (2005).

32. K. Yoshikawa, K. Adachi, M. Nishijima, T. Takadera, S. Tamaki, K.-I. Harada, K. Mochida and H. Sano, B-Cyanoalanine Production by Marine Bacteria on Cyanide-Free Medium and Its Specific Inhibitory Activity toward Cyanobacteria, Appl. Environ. Microbiol., **66(2)**, 718-722 (2000).

33. B. Zhang, G. Cai, H. Wang, D. Li, X. Yang, X. An, X. Zheng et al., Streptomyces Alboflavus Rps and Its Novel and High Algicidal Activity against Harmful Algal Bloom Species Phaeocystis Globosa, PloS one, **9(3)**, e92907 (2014).

34. Q.-q. Zhang, S. H. Lei, X. L. Wang, L. Wang, P. Yu, Y. W. Chen, X. Y. Zeng et al., Research on Discrimination of 3d Fluorescence Spectra of Phytoplanktons, Spectroscopy and Spectral Analysis, **24(10)**, 1227-1229 (2004).