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The Potential of Graphene Oxide as Antimicrobial Agent against Pathogenic Bacteria Isolated From Aquaculture Sites.

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ABSTRACT

This paper described the potential of graphene oxide as antimicrobial agent against pathogenic bacterial isolates from aquaculture sites. The increasing of antibiotic resistance case among pathogenic bacteria from aquaculture sites led to the most of the commercial antibiotics no longer effective in controlling bacterial diseases infected in aquaculture species. Hence, this situation urged fish farmer in seeking new antimicrobial agent to alternate commercial antibiotics. In the present study, antimicrobial property of graphene oxide against a total of 31 bacterial isolates was reveal through two fold broth microdilution method with kanamycin as positive control. The minimum inhibitory concentration (MIC) values of graphene oxide against the tested bacteria were ranged from 7.81 to 125 mg/L. The finding of the present study showed the huge potential of graphene oxide as antimicrobial agent in controlling pathogenic bacteria from aquaculture species. Further study should be carried out before the finding can come to a commercial sense.
Keywords: graphene oxide; antimicrobial; bacteria; aquaculture

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INTRODUCTION

Disease outbreak is recognized as a main constraint to the development of aquaculture in world wide (Lee et al., 2009a). Most of diseases were reported can devastate whole aquaculture farm and may lead to the farmer bankruptcy. Bacterial disease is one of the disease was found threaten to most of the aquaculture species (Lee et al., 2009b). The famous bacteria that pose a threat to aquatic animal health are *Aeromonas hydrophila*, *Edwardsiella tarda*, *Streptococcus* sp., *Vibrio harveyi*, *V. alginolyticus* and *V. parahaemolyticus*. Hence, there is must to seek an effective antimicrobial agent in order to overcome the arise problem.

Till present, antibiotic is the only solution as antimicrobial agent either for treatment or prophylactive agent against diseases infected farmed aquatic organisms. However, antibiotic was no longer effective in controlling diseases due to misuse or overuse among farmer in handling aquatic animal health. Recently, the incidence of antibiotic resistance case among pathogenic bacteria is increasing rapidly and farmers were left with no option and continue to use antibiotic (Lee et al., 2010). Thus it is a must to find alternative antimicrobial agent for aquaculture uses.

Graphene oxide (GO) is reported possess antimicrobial property against various microbial. There are many reports revealed the inhibitory activity of GO against bacteria in the literature. For instance, in the study of Liu et al. (2011) revealed that there are three steps in the mechanism of antimicrobial activity of graphene oxide towards *Escherichia coli*. Other study that revealed the antimicrobial property of graphene oxide is Manash et al. (2011) claimed that GO showed inhibitory activity against *E. coli* and *Pseudomonas aeruginosa*. However, in so far no study was recorded the potential of GO in controlling pathogenic bacteria from aquatic animals. Hence, this study was carried to investigate antimicrobial activity of GO against bacteria isolated from various of aquatic animals.

MATERIALS AND METHODS

Bacterial isolates

A total of 31 bacterial isolates was provided by Aquaculture Research Lab, Universiti Malaysia Kelantan Jeli Campus. They were *Aeromonas hydrophila* (n = 6), *Edwardsiella tarda* (n = 7), *Flavobacterium* sp. (n = 1), *Pseudomonas* sp. (n = 3), *Streptococcus* sp. (n = 5), *Vibrio harveyi* (n = 3), *V. parahaemolyticus* (n = 3), *Vibrio alginolyticus* (n = 3). The bacterial isolates were cultured in tryptic soy broth (TSB) (Merck, Germany) for 24 h at room temperature. The concentration of the bacterial culture was adjusted to 10^9 CFU/ ml and cross check with a ELISA reader (Bio Rad, USA) before carried out antimicrobial test (Lee et al., 2011a).

Graphene oxide (GO) preparation

Oxidation of graphite was carried out by mixing H_2SO_4:H_3PO_4 (320:80 mL), graphite flakes and $KMnO_4$ (18g) using a magnetic stirrer. After adding all the materials slowly, the one-pot mixture was left for stirring for 3 days to allow the oxidation of graphite. The colour of the mixture was changed from dark purplish green to dark brown. Later, H_2O_2 solution was added to stop the oxidation process, and the colour of the mixture was changed to bright yellow, indicating a high oxidation level of graphite. The graphite oxide formed was washed three times with 1 M of HCl aqueous solution and repeatedly with deionized water until a pH of 4-5 is achieved. The washing process was carried out using simple decantation of supernatant via a centrifugation technique with a centrifugation force 10,000 g. During the washing process with deionized water, the graphite oxide experienced exfoliation, which was resulted in the thickening of the graphene solution, forming a GO gel (Victor et al., 2014).

Minimum Inhibitory Concentration (MIC) values determination

Minimum inhibitory concentration (MIC) values were determined using two fold micro broth dilution method in 96-wells microliter plate format. Bacterial suspensions were inoculated into wells in the presence of graphene oxide with concentration start from 0.244 to 500 mg/L and positive control, kanamycin (Lee et al., 2011b). The growth of bacteria were checked after 24 h (s) incubation. MIC value is determined as the lowest concentration of antimicrobial agent inhibits the visible growth of the inoculated bacteria (Lee et al., 2011c).

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RESULTS

Table 1: Minimum Inhibitory Concentration (MIC) values of graphene oxide against pathogenic bacterial species isolated from aquaculture sites.

Bacteria species	Sources	Minimum Inhibitory Concentration (mg/L) of Graphene Oxide	Minimum Inhibitory Concentration (mg/L) of Kanamycin
Aeromonas hydrophila	Red Hybrid Tilapia, *Oreochromis* spp.	125	62.5
Aeromonas hydrophila	African catfish, *Clarias gariepinus*	31.25	125
Aeromonas hydrophila	Black Tilapia, *Oreochromis niloticus*	62.5	15.63
Aeromonas hydrophila	Climbing Perch, *Anabas testidineus*	7.81	62.5
Aeromonas hydrophila	Asian Seabass, *Lates calcarifer*	15.63	125
Aeromonas hydrophila	Asian Seabass, *Lates calcifer*	31.25	62.5
Edwardsiella tarda	African catfish, *Clarias gariepinus*	7.81	31.25
Edwardsiella tarda	African catfish, *Clarias gariepinus*	62.5	62.5
Edwardsiella tarda	Black Tilapia, *Oreochromis niloticus*	7.81	31.25
Edwardsiella tarda	Climbing Perch, *Anabas testidineus*	125	125
Edwardsiella tarda	American Bullfrog, *Rana catesbeina*	125	125
Edwardsiella tarda	American Bullfrog, *Rana catesbeina*	62.5	31.25
Edwardsiella tarda	American Bullfrog, *Rana catesbeina*	31.25	62.5
Flavobacterium spp.	Black Tilapia, *Oreochromis niloticus*	7.81	31.25
Pseudomonas sp.	Climbing Perch, *Anabas testidineus*	15.63	31.25
Pseudomonas sp.	African catfish, *Clarias gariepinus*	62.5	125
Pseudomonas sp.	African catfish, *Clarias gariepinus*	7.81	62.5
Streptococcus sp.	Red Hybrid Tilapia, *Oreochromis* sp.	31.25	125
Streptococcus sp.	Red Hybrid Tilapia, *Oreochromis* sp.	62.5	62.5
Streptococcus sp.	Red Hybrid Tilapia, *Oreochromis* sp.	15.63	7.81
Streptococcus sp.	Red Hybrid Tilapia, *Oreochromis* sp.	31.25	15.63
Streptococcus sp.	Red Hybrid Tilapia, *Oreochromis* sp.	15.63	7.81
Vibrio alginolyticus	Asian Seabass, *Lates calcifer*	7.81	31.25
Vibrio alginolyticus	Blood Cockle, *Anadara granosa*	62.5	15.63
Vibrio alginolyticus	Blood Cockle, *Anadara granosa*	15.63	7.81
Vibrio harveyi	Black Tiger Shrimp, *Penaeus monodon*	62.5	31.25
Vibrio harveyi	Blood Cockle, *Anadara granosa*	125	15.63
Vibrio harveyi	Blood Cockle, *Anadara granosa*	7.81	62.5
Vibrio parahaemolyticus	White Leg Shrimp, *Litopenaeus vannamei*	31.25	62.5
Vibrio parahaemolyticus	White Leg Shrimp, *Litopenaeus vannamei*	62.5	15.63
Vibrio parahaemolyticus	White Leg Shrimp, *Litopenaeus vannamei*	15.63	31.25

In the present study, graphene oxide (GO) has been successfully produced through the above mentioned method. The GO was subjected to MIC test against 31 bacterial isolates from aquaculture sites. The MIC values of GO against the tested bacterial isolates were ranged from 7.81 to 125 mg/L whereas MIC values of kanamycin were 7.81 to 125 mg/L. The MIC values of GO against *Aeromonas hydrophila* isolated from Red Hybrid Tilapia, *Oreochromis* spp., African catfish, *Clarias gariepinus*, Black Tilapia, *Oreochromis niloticus*, Climbing Perch, *Anabas testidineus* and Asian Seabass, *Lates calcifer* were ranged from 7.81 to 125 mg/L. *Edwardsiella tarda* isolated from African catfish, *Clarias gariepinus*, Black Tilapia, *Oreochromis niloticus*, Climbing Perch, *Anabas testidineus* and American Bullfrog, *Rana catesbeina* can be inhibited by GO with the range 7.81 to 125 mg/L whereas GO showed the lowest MIC value against the only one bacterial isolate of *Flavobacterium* spp. from Black Tilapia. In the present study, three bacterial isolates of *Pseudomonas* spp. obtained from Climbing Perch, *Anabas testidineus* and African catfish, *Clarias gariepinus* showed no growth at 7.81, 15.63 and 62.5 mg/L of GO. The growth all bacterial isolates of *Streptococcus* sp. isolated from red hybrid tilapia can be inhibited GO at the concentration of 15.63 to 62.5 mg/L. All bacterial isolates of *Vibrio* species of the present study showed inhibition activity against GO at concentration of ranging from 7.81 to 125 mg/L. *Vibrio* species that applied in the present study were *Vibrio harveyi*, *V. parahaemolyticus* and *V. alginolyticus* where isolated from Asian Seabass, *Lates calcarifer*, Blood Cockle, *Anadara granosa*, Black Tiger shrimp, *Penaeus monodon* and White Leg Shrimp, *Litopenaeus vannamei*.

DISCUSSION

In the present study, grapheme oxide was successfully generated and showed antimicrobial activity against all the tested bacteria. Till to date, a lot of studies have been documented and recorded about antimicrobial activity of GO against various type of microorganisms. For example, Chen et al. (2014) claimed GO showed inhibitory activity against plant pathogens, fungi and bacteria. The study was revealed the mechanism of the GO by causing cell lysis to the fungi and bacteria. Liu et al. (2011) showed that graphene oxide possess antimicrobial activity toward *Escherichia coli*. They make comparison antimicrobial activity of graphite, graphite oxide, grapheme oxide and reduce graphene oxide against *E. coli*. They found that GO showed the best results in inhibiting E. coli. Another study of Gurunathan et al. (2012) was also revealed GO showed better result inhibiting *P. aeruginosa* compared to reduced graphene oxide. Other study of Satish et al. (2013) claimed that GO can inhibit the growth of *Klebsiella* and *Staphylococcus*. Although antimicrobial activity of GO has been widely documented but to our knowledge the database information of antimicrobial activity of GO against bacteria isolated from aquaculture sites of the present study was firstly reported.

Application of graphene oxide as antimicrobial agent has been widely recorded. The GO was developed into antimicrobial materials. For instance, Manash et al. (2011) found that graphene oxide sheet can inhibit *E. coli* and *P. aeruginosa*. Other use of GO as antimicrobial agent is material in food packaging. Based on the literature survey, GO has been widely used in many industries. One of the industry is pharmaceutical. GO was found able to be as material for biosensor detector (Zhu et al., 2010), drug carrier and assisting in curing cancer disease where it may help to drug only targeted to tumor cells not to healthy cells. GO was also reported low toxicity where has no effect to the health. In spite of the fact, GO can considered as a new candidate as antimicrobial agent to alternate the available commercial antibiotic for aquaculture uses. Hence, the finding of the present study revealed that GO alone can inhibit the growth of various bacterial species from aquaculture sites may justify and support the application of GO for aquaculture uses.

The increasing of antibiotic resistance case among pathogenic bacteria from aquaculture sites due to extensive used of commercial antibiotic in managing aquatic animal health led to the most of the antibiotic was no longer effective in controlling bacterial diseases (Lee et al., 2013). Therefore it is a must to find an alternative antimicrobial agent for aquaculture uses. Furthermore, the application of antibiotic in aquaculture may pose a threat to public health and environmental (Lee et al., 2012). Hence, this study was successfully documented inhibitory activity of self-made GO against various types of bacteria. However, furthermore study should be carried out in the near future before the application of GO in aquaculture can come to a commercial sense.

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REFERENCES

[1] Chen, J; Peng, H; Wang, X; Shao, F; Yuan, Z and Han, H (2014). Graphene oxide exhibits broad-spectrum antimicrobial activity against bacterial phytopathogens and fungi conidia by intertwining and membrane perturbation. Nanoscale (3) 6: 1879-1889.

[2] Gurunathan, S; Han, JW; Ahmed, AD; Vasuki, E; Park, MR; Kwon, DN and Kim, JH (2012). Antibacterial activity of dithiothreitol reduced grapheme oxide. J Industrial Engin Chem 19 (4): 1280-1288.

[3] Krishnamoorthy, K; Umasuthan, N; Mohan, R; Lee, J and Kim SJ (2012). Antibacterial activity of graphene oxide nanosheets. Sc. Advan. Mat. 4 (11): 1111-1117.

[4] Krishnamoorthy, K; Umasuthan, N; Mohan, R; Lee, J and Kim SJ (2012). Graphene oxide nanostructures modified multifunctional cotton fabrics. Applied Nanoscience 2 (2): 119-126.

[5] Lee, SW; Najiah, M; Wendy, W; Zahrol, A and Nadirah, M (2009a). Multiple antibiotic resistance pattern and heavy metal resistance profile of bacteria isolated from Giant Freshwater Prawn, *Macrobrachium rosenbergii*, hatchery. Agric Sc in China 8 (6): 740-745.

[6] Lee, SW; Najiah, M; Wendy, W; Nadirah, M and Faizah, SH (2009b). Occurrence of heavy metals and antibiotic resistance in bacteria from internal organs of American bullfrog, *Rana catesbeina*, raised in Malaysia. J Venomous Anim Tox include Trop Dis 15 (2): 353-358.

[7] Lee, SW; Najiah, M; Wendy, W and Nadirah, M (2010). Antibiogram and heavy metal resistance of pathogenic bacteria isolated from moribund cage cultured silver catfish, *Pangasius sutchi* and red hybrid tilapia, *Oreochromis* spp. Front Agric China 4 (1): 116-120.

[8] Lee, SW; Wendy, W; Julius, YFS and Desy, FS (2011a). Antioxidant, antimicrobial, anticancer property and chemical composition of different parts (corm, stem and leave) of *Colocasia esculenta* extracts. Annales Universitatis Mariae Curie-Sklodowska, Sectio DDD: Pharmacia 24 (3): 9-16.

[9] Lee, SW; Wendy, W; Julius, YFS and Desy, FS (2011b). Characterization of antioxidant, antimicrobial, anticancer property and chemical composition of *Michelia champaca* seed and flower extracts. Stamford J Pharma Sc. 4(1): 19-24.

[10] Lee, SW; Wendy, W; Julius, YFS and Desy, FS (2011c). Characterization of antioxidant, antimicrobial, anticancer property and chemical composition of *Peperomia pellucida* leaf extracts. Acta Medica Iranica 49 (10): 670-674.

[11] Lee, SW; Wendy, W; Julius, YFS and Desy, FS (2012). Characterization of antioxidant, antimicrobial, anticancer property and chemical composition of *Phyllanthus urinaria* Linn. leaf extract. Pharmacologyonline 2: 36-40.

[12] Lee, SW and Wendy, W (2013). Chemical composition and antimicrobial activity of *Cymbopogon nardus* citronella essential oil against systemic bacteria from aquatic animals. Iranian J Microbiol 5 (2): 147-152.

[13] Liu, S; Zeng, TH; Hofmann, M; Burcombe, E; Wei, J; Jiang, R; Kong, J and Chen, Y (2011). Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced graphene oxide: membrane and oxidative stress. ACS Nano 5 (9): 6971-6980.

[14] Manash, RD; Rupak, KS; Ratul, S; Vinayak, SK; Manjusha, VS and Pinaki, S (2011). Synthesis of silver nanoparticles in an aquaeous suspension of grapheme oxide sheets and its antimicrobial activity. Colloids and surfaces B: Biointerfaces 83: 16-22.

[15] Satish, B; Venkateswara, RK; Shilpa, CCH and Tejaswi, T (2013). Synthesis and characterization of graphene oxide and its antimicrobial activity against *Klebsiella* and *Staphylococus*. Int J Adv Biotech Res 4 (1): 142-146.

[16] Victor, C; Higgins, D; Yu, A; Xiao, X; Chen, Z and Zhang, J (2014). A review of graphene and graphene oxide sponge: material synthesis and applications to energy and the environment. Energy Environ. Sc. 5 (7): 1564-1596.

[17] Zhu, Y; Murali, S; Cai, W; Li, X; Suk, JW; Potts, JR and Ruoff, RS (2010). Graphene and graphene oxide: synthesis, properties and applications. Adv. Mat. 1 – 19.