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Research Article

Cobalamin and Thiamine Effect on Microalgae Biomass Production in the Glagah Consortium

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ABSTRACT
The Glagah consortium is a mixed culture of various microalgae and bacteria isolated from Glagah Beach, Yogyakarta. Cobalamin and thiamine, which are given by symbiotic bacteria, are assumed will increase biomass. This study aimed to determine the effect of cobalamin and thiamine on microalgae biomass production in the Glagah consortium. The microalgae of Glagah consortium were cultivated for 10 days with vancomycin and gentamicin antibiotic as treatment and without antibiotics as a control. The parameters measured included the number of bacterial colonies, cobalamin and thiamine levels measured by LC-MS, chlorophyll a and b levels, cell density of microalgae and dry biomass. The highest level of cobalamin and thiamine was in the Glagah consortium without antibiotics. Cobalamin and thiamine increased in the exponential phase along with the increasing *Staphylococcus* sp. colonies. The Quantity of *Staphylococcus* sp. colonies in the exponential phase was 62.10^5 (cfu/mL). The level of cobalamin in the exponential phase was 2.33 µg/L and the level of thiamine in the exponential phase was 49.71 µg/L. The highest productivity dried weight biomass was 0.0134 g/L/day in the day-6th on the Glagah consortium without antibiotics. This result showed that microalgae and bacterial interaction was mutualism symbiosis involving cobalamin and thiamine that increased in the exponential phase along with the increasing *Staphylococcus* sp. colonies. This interaction was able to increase biomass microalgae.

INTRODUCTION
Microalga is a micro-sized algae which produces very useful biomass and it was utilized to fulfil human's needs in their daily lives, such as biofuel, the pharmaceutical industries, and the supplement of food. Biomass produced by microalga consortium which involves the interaction of microalgae and bacteria has not been well researched. The beneficial interaction between microalgae and bacteria is assumed to improve the production of biomass. One natural example of how microalgae and bacteria interact is the Glagah consortium isolated from Glagah beach, Yogyakarta. The consortium of Glagah is a mixed culture consisting of various species of microalgae and some symbiotic bacteria. Suyono et al. (2018) reported that the mixed culture of Glagah consortium which consists of *Cyclotella polymorpha*, *Cylindrospermopsis raciborskii*, *Golenkinia radiata*, *Sy-*

racosphaera pirus, *Corethron criophilum*, *Cochliopodium vestitum* and *Chlamydomonas* sp. have a mutualism symbiosis with symbiotic bacteria.

The association of symbiotic bacteria and microalgae in the consortium of Glagah is a mutualism symbiosis where microalgae and bacteria support each other for their growths (Suyono et al. 2018). Generally, microalgae as a photoautotroph organism which is able to use the energy of the light in changing an inorganic carbon becomes an organic carbon, expanding the source of carbon to support the growth of bacteria and used for the synthesis of DNA, and improving bacteria's biofilm (Matsui et al. 2003). In that kind of symbiosis, as the reciprocal relation, bacteria support the growth of microalgae by producing CO_2 and use the excess of oxygen produced by microalgae, so it can prevent the photorespiration which is harmful for the microalgae also the inorganic nutrition and the growth factor which is needed (Amin et al. 2015; Wang et al. 2016)

Based on the analysis of the type of microalgae in the consortium of Glagah, cobalamin (B12) and thiamine (B1) are vitamins B which are expected to be significantly involved in the relation of mutualism symbiosis between microalgae consortium of Glagah and its symbiotic bacteria. Some kinds of microalgae consortium of Glagah are indicated to need the cobalamin and thiamine, but they are unable to synthesise themselves (auxotroph). According to Croft et al. (2006), the auxotroph microalgae to cobalamin and thiamine are in the group of Chlorophyta and Haptophyta. *Golenkinia radiata* and *Chlamydomonas* sp. which are microalgae in the consortium of Glagah include the group of Chlorophyta. *Syracosphaera pirus* is microalgae in the consortium of Glagah which is auxotroph to the cobalamin and thiamine and both of those vitamins obtained from their symbiosis. Microalgae auxotroph to the cobalamin and thiamine need the cobalamin and thiamine from another microbes. Cobalamin and thiamine are vitamins B that are only produced by prokaryotic organisms, especially bacteria (Warren et al. 2002)

Cobalamin and thiamine given by symbiotic bacteria are assumed to be able to support the growth of microalgae due to the function of these two vitamins. Cobalamin which plays an important role in protein synthesis, and thiamine which plays an important role in the formation of amino acids and carbohydrates can increase cell growth and division because cell growth is controlled by these compounds (Konopka et al. 2015; Rosnow et al. 2018). So, the presence of bacteria is assumed to be able to increase microalgae cell density and its biomass. Therefore, the research about the interaction of bacteria and microalgae in the Glagah consortium to increase biomass is needed to prove this.

The goal of this research was to determine the effect of cobalamin and thiamine on microalgae biomass levels in the Glagah consortium. The interaction of microalgae and symbiotic bacteria in the Glagah consortium involving cobalamin and thiamine in the production of microalgae biomass was studied by analysing the number of bacteria colonies, the level of cobalamin, thiamine, chlorophyll a, b and dry biomass in Glagah consortium without antibiotics and with antibiotics. The antibiotics used are Vancomycin and Gentamicin to inhibit the growth of positive gram bacteria and the negative gram bacteria in the consortium of Glagah (Li et al. 2011; Grenni et al. 2018)

MATERIALS AND METHODS

Materials

The materials of this research included microalgae consortium of Glagah isolated from the Glagah beach, Yogyakarta, gentamicin and vancomycin antibiotics, BBM (Bold Basal Medium) for cultivation, NA (Nutrient

Agar) medium, methanol, the vitamin standard of cobalamin and thiamine.

Methods

This research consisted of three treatments. The first treatment was the Glagah consortium given 25 ppm Gentamicin and 100 ppm Vancomycin antibiotics. In the treatment 2, the antibiotic dosage used was 50 ppm Gentamicin and 200 ppm Vancomycin. In the treatment 3, the dosage of antibiotic used was 100 ppm Gentamicin and 400 ppm Vancomycin. The control in this research was the consortium of Glagah without antibiotics. Every treatment consisted of three repetitions.

The cultivation of the Glagah Consortium

First, 250 mL of the consortium of Glagah was mixed into the 250 mL BBM (Bold Basal Medium) in the 1000 mL culture bottle. After that, it was given some serials of treatment for 10 days. In the treatment 1, the dosage of antibiotic used was 25 ppm of Gentamicin and 100 ppm of Vancomycin. In the treatment 2, the dosage of antibiotic used was 50 ppm of Gentamicin and 200 ppm of Vancomycin. In the treatment 3, the dosage of antibiotic used was 100 ppm Gentamicin and 400 ppm Vancomycin. A control in this study was microalgae consortium of Glagah without antibiotics. Each treatment needed 3 culture bottles as the repetition. The lighting was conducted by using TL lamp continuously, cool white fluorescent and was continuously given an aeration.

The Calculation of Microalgae Cell

The calculation of microalgae cells was conducted every day from the day -0 until the day-10th by using a microscope set by Opti Lab and Haemocytometer Neubauer. The sample used was 1 mL then it was entered into the tube 2 mL, then it was counted using a microscope. The calculation was conducted by counting the cell at the two view fields (5 sides) at Haemocytometer Neubauer. The formulation of the cell density as follow:

Cell density (the quantity of cell/mL) = (the quantity of counted cell)/64 x 160 x 10^4 x 1.25

The Calculation of the Number of Bacterial Colonies

Calculation of the number of bacterial colonies was done by the spread plate method. The medium used was NA (Nutrient Agar) medium with pH 6-7. The liquid NA medium was poured into the petri dish aseptically and waited for it to dry. After drying, 6 dilutions of the Glagah consortium culture sample were carried out by mixing 0.1 mL of the sample with 0.9 BBM medium for 10^{-1} dilution, then 0.1 mL of the mixture was taken and mixed with 0.9 BBM medium for dilution 10-2 and so on until dilution 10^{-6}. The 10^{-4}, 10^{-5} and 10^{-6} dilutions were poured into each NA medium then were flatten with driglaski. Spread plates were calculated at early, log, and stationary phase. After 48 hours incubation at room temperature, the colon counted with a hand counter. The Total Plate Count was calculated according to SNI 2897: 2008 concerning the method of testing microbial contamination in meat, eggs and milk, and its processed products.

The Calculation of Dry Biomass

The calculation of the dry biomass was conducted every day from day-0 until day 10^{th}. First, 2 mL of the sample was entered into the tube, then it was centrifuged with the speed 8000 rpm for 15 minutes. Supernatant was excluded and left the pellet by filtering with the filter paper. The re-

sult of the filter was wrapped with the filter paper, then it was entered into the oven for 15 minutes in 70°C.

The Calculation of Chlorophyll a and b

2 mL of sample was centrifuged at a speed of 12000 rpm for 10 minutes, then pellet was added by 1 mL of methanol, then it was centrifuged at a speed of 12000 rpm for 10 minutes (J. Cheng et al. 2016). The sample poured into a glass cuvette and the absorbance was calculated at wavelengths of 480, 652 and 665 nm in the spectrophotometer. Chlorophyll a and b levels were calculated using the following equation.

Chlorophyll a (mg/L) = 16.5169 x A665 - 8.0962 x A652
Chlorophyll b (mg/L) = 27.4405 x A652 - 12.1688 x A665

The Calculation of Cobalamin and Thiamine

The level of cobalamin (Vitamin B12) and thiamine (Vitamin B1) were counted by using LC-MS. The sample taken was 10 mL and was obtained from three phases of the growth of microalgae that was early, log and stationary phase. First, the preparation of the sample was conducted by filtering the sample with millex 0.22 µM, afterwards injected 2 µL to the LCMSMS. Second, vitamin standard was made by weighing the vitamin standard of B1 and vitamin B12 1000 ppm for each, then made the standard of the mixture containing 500 ppm, made the standard of mixture series of 25 ppb, made the standard of mixture series of 2 ppb afterwards injected 2 µL to LCMSMS duplo.

RESULTS AND DISCUSSION

The consortium of Glagah was a mixture culture isolated from Glagah Beach, Special Region of Yogyakarta which consisted of various species of microalgae and symbiotic bacteria. Suyono et al. (2018) reported that the mixture culture of microalgae Glagah consisted of *Cylindrospermopsis raciborskii*, *Syracosphaera pirus*, *Corethron criophilum*, *Golenkinia radiate*, *Cochliopodium vestitu*, *Cyclotella polymorpha* and *Chlamydomonas* sp. had a relation of mutualism symbiosis with the symbiotic bacteria. The symbiotic bacteria successfully cultured in this research were 4 species, they were: *Clostridium* sp., *Streptococcus* sp., *Veillonella* sp., dan *Staphylococcus* sp.

The injection of Vancomycin and Gentamicin antibiotics was able to decrease the quantity of the bacteria's colony which had symbiosis with microalgae. It could be seen from the calculation of the quantity of the colony of 4 species bacteria observed at the 3 points of the growth phase of microalgae, those were early, log, and stationary phase (Table 1).

Table 1. The quantity of bacteria's colony at the growth of microalgae Glagah consortium phase in every treatment.

The Quantity of Bacteria's Colony (cfu/mL)	Control (Without Antibiotic)			Treatment 1 (100 ppm Vancomycin, 25 ppm Gentamicin)			Treatment 2 (200 ppm Vancomycin, 50 ppm Gentamicin)			Treatment 3 (400 ppm Vancomycin, 100 ppm Gentamicin)		
	Early	Log	End	Early	Log	End	Early	Log	End	Early	Log	End
Staphylococcus sp	34. 10^5	62. 10^5	44. 10^5	22. 10^5	16. 10^5	<100	1,7. 10^5	<100	<100	2,3. 10^5	<100	<100
Streptococcus sp.	300. 10^5	194. 10^5	505,6. 10^5	14. 10^5	<100	<100	40. 10^5	<100	<100	<100	<100	<100
Clostridium sp.	41. 10^5	1,85. 10^5	4. 10^5	22. 10^5	16. 10^5	<100	1,7. 10^5	<100	<100	<100	<100	<100
Veillonella sp.	4,5. 10^5	3. 10^5	4. 10^5	2. 10^5	<100	<100	80. 10^5	<100	<100	<100	<100	<100

Based on the analysis of the species of bacteria, only *Staphylococcus* sp. was able to produce cobalamin and thiamine. *Staphylococcus* sp. efficiently gave cobalamin and thiamine in the exponential phase of the growth of microalgae in the culture of Glagah consortium without the antibiotic. In the exponential phase, the number of *Staphylococcus* sp. colony was increasing and in the stationary phase was decreasing (Table 1). It was in line with the level of thiamine which was increasing in the exponential phase whereas in the stationary phase was decreasing (Table 2). Table 2 showed that in the control, the level of thiamine was increasing from 0.41 µg/L in the early phase to 49.71 µg/L in the exponential phase, whereas in the stationary phase it was decreasing to 0.44 µg/L.

Therefore, it was assumed that the *Staphylococcus* sp. affected the increasing of thiamine*'s* quantity. *Staphylococcus* sp. was able to synthesise thiamine because it contained the biosynthesis coded thiamine gene (Müller et al. 2009). The level of cobalamin was also increasing at the exponential phase, that was from 1.21 µg/L at the early phase became 2.33 µg/L (Table 2). Besides affecting the level of thiamine at the exponential phase, *Staphylococcus* sp. also affecting the level of cobalamin. *Staphylococcus* sp. had the enzyme which was involved in the cobalamin*'s* biosynthesis (Leisico et al. 2018).

Staphylococcus sp. as the provider of cobalamin and thiamine was also working together with another bacterium which were *Streptococcus* sp., *Clostridium* sp., and *Veillonella* sp. in supporting the growth of microalgae. The culture of the Glagah consortium without antibiotics had more various quantities of the bacteria's colony than the consortium of Glagah with antibiotics so the growth of microalgae was more efficient as seen from the cell density, chlorophyll a, b and biomass. The *Streptococcus* sp. and *Staphylococcus* sp. were facultative anaerobe produced CO_2 by utilising the excess of oxygen produced by microalgae so it inhibited the photorespiration which harmed the microalgae (Wang et al. 2016). It also provided of nutrition in the form of nitrogen and phosphorus needed for the growth of microalgae (Jiang et al. 2007; Foster et al. 2011; Kazamia et al. 2012; Wang et al. 2016). Bacteria can produce the factors of growth such as chelator, phytohormone (IAA) to support the growth of microalgae (Amin et al. 2015; Wang et al. 2016). *Clostridium* sp. can produce Indoleacetic acid (IAA) which was synthesised from the tryptophan which was produced by microalgae and then that IAA was used by microalgae to support their growth (Whitehead et al. 2008; Cooper & Smith 2015).

The calculation of cell density showed that Glagah consortium culture with the highest quantity of *Staphylococcus* sp. colonies and the highest levels of cobalamin and thiamine had the highest cell density of microalgae (Figure 1). It was because cobalamin functioned as a cofactor at the synthesis of methionine which was the factor of initiation translation/ synthesis of protein so all expressions of gen depended on those

Table 2. The level of cobalamin and thiamine at the phase of microalgae Glagah consortium growth in every treatment.

Measurement	Control (Without Antibiotic)			Treatment 1 (100 ppm Vancomycin, 25 ppm Gentamicin)			Treatment 2 (200 ppm Vancomycin, 50 ppm Gentamicin)			Treatment 3 (400 ppm Vancomycin, 100 ppm Gentamicin)		
	Early	Log	End	Early	Log	End	Early	Log	End	Early	Log	End
The level of thiamine (µg/L)	0.41	49.71	0.44	1.23	1.13	0.43	0.73	0.57	0.39	1	0.78	0.46
The level of cobalamin (µg/L)	1.21	2.33	0.72	1.02	0.94	0.63	1.09	1.02	0.67	1.74	1.08	0.63

vitamins (Croft et al. 2006). Besides that, thiamine was a micronutrient needed as a cofactor of some enzymes which were involved in the central metabolism of the formation of amino acid and carbohydrate that was in the form of glycolysis process, Krebs' cycle, phosphate pentose path, and the Calvin's cycle (Moulin et al. 2013)

The quantity of the highest bacterium's colony in the exponential phase at the control sample caused the high level of cobalamin at that phase. The higher level of cobalamin in the control also affected the level of chlorophyll a and chlorophyll b. Based on the measurement result of chlorophyll level a and b showed that the chlorophyll a and b at the consortium of Glagah without antibiotics (control) was higher than the consortium of Glagah with the antibiotics (treatment) (Table 3,4 and 5). Cobalamin was directly involved in the photosynthesis process because it functioned as a photoreceptor which was able to respond the light for the activation of photosynthetic genes expression (Z. Cheng et al. 2016). The received light converted the form of adenosylcobalamin to OHB12 which then activated photosynthetic gene expression. Besides the function of cobalamin produced by bacteria, bacteria also created good photosynthetic conditions by consuming oxygen produced by microalgae thereby reducing the excess oxygen which prevented photorespiration (Z. Cheng et al. 2016).

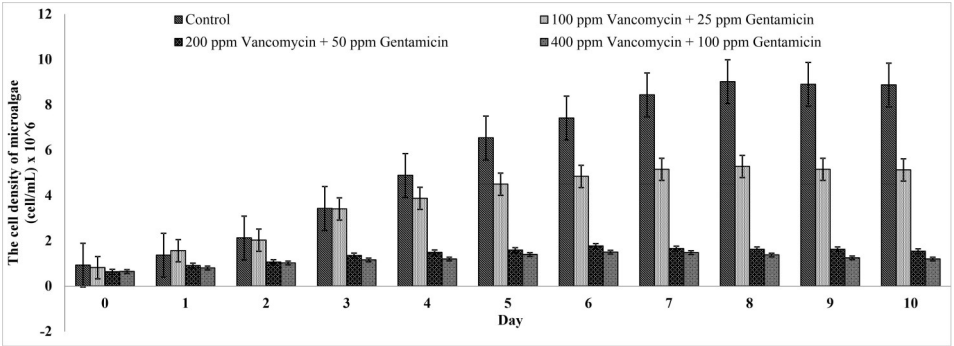

Figure 1. The Cell Density of the Microalgae Glagah Consortium.

The result of the cell density of microalgae was directly proportional with their dry weight (Figure 2). It was because cobalamin played an important role in protein synthesis, and thiamine which played an important role in the formation of amino acids and carbohydrates can increase cell growth and division because cell growth was controlled by these compounds so microalgae biomass (Konopka et al. 2015; Rosnow et al. 2018).

The highest levels of chlorophyll a and b in Glagah consortium was also impacted in their dry biomass. Chlorophyll a and b were photosynthetic pigments that absorb light with certain wavelengths for photosynthesis. Photosynthesis processes that occur properly increased the ability to transform light energy into carbon source for microalgae growth so that biomass increased. The increasing of chlorophyll b also affected the molecular organisation of thylakoid membranes because it functions as an antenna complexes in thylakoid membranes (Voitsekhovskaja & Tyutereva 2015).

The highest peak of the dry biomass was on the day-6th with the highest productivity in the control of 0.01341 g/L (Table 6). The dry biomass showed the quantity of biomass which could be used to fulfil the needs of humans. This research showed that the interaction of the microalgae Glagah consortium with symbiotic bacteria was able to increase the level of cobalamin and thiamine and these vitamins influenced the increasing of chlorophyll a, b and dry biomass level.

Table 3. The level of chlorophyll a, b and in the early phase.

SAMPLE	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)
Control (without antibiotics)	2.590	3.080
Treatment 1 (100 ppm Vancomycin, 25 ppm Gentamicin)	3.084	3.433
Treatment 2 (200 ppm Vancomycin, 50 ppm Gentamicin)	3.172	4.462
Treatment 3 (400 ppm Vancomycin, 100 ppm Gentamicin)	2.486	2.811

Table 4. The level of chlorophyll a, b in the log phase.

SAMPLE	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)
Control (without antibiotics)	17.219	16.721
Treatment 1 (100 ppm Vancomycin, 25 ppm Gentamicin)	15.154	15.675
Treatment 2 (200 ppm Vancomycin, 50 ppm Gentamicin)	7.054	8.332
Treatment 3 (400 ppm Vancomycin, 100 ppm Gentamicin)	5.830	6.350

Table 5. The level of chlorophyll a, b in the stationary phase.

SAMPLE	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)
Control (without antibiotics)	16.242	6.880
Treatment 1 (100 ppm Vancomycin, 25 ppm Gentamicin)	11.824	6.198
Treatment 2 (200 ppm Vancomycin, 50 ppm Gentamicin)	7.443	5.803
Treatment 3 (400 ppm Vancomycin, 100 ppm Gentamicin)	5.748	4.780

Table 6. The productivity of dry weight biomass.

SAMPLE	Productivity of dry weight biomass (g/L/day) x 10^{-2}
Control (without antibiotics)	1.34
Treatment 1 (100 ppm Vancomycin, 25 ppm Gentamicin)	1.02
Treatment 2 (200 ppm Vancomycin, 50 ppm Gentamicin)	0.92
Treatment 3 (400 ppm Vancomycin, 100 ppm Gentamicin)	0.75

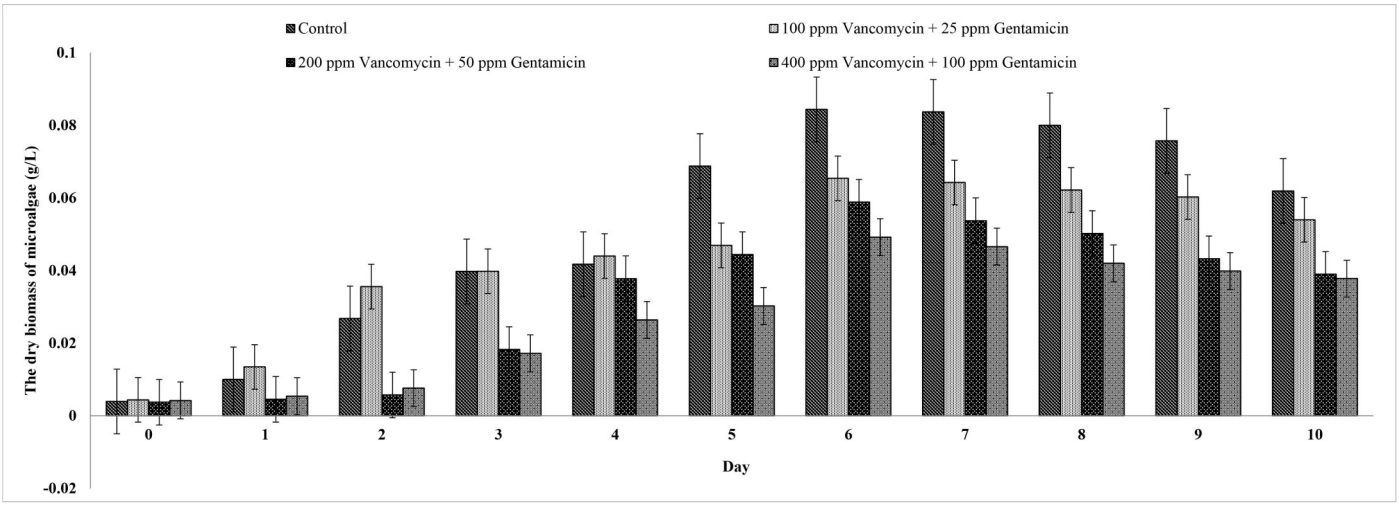

Figure 2. The Dry Biomass of the Microalgae Glagah Consortium.

CONCLUSIONS

This research showed that microalgae Glagah consortium consisting of *Cyclotella polymorpha*, *Cylindrospermopsis raciborskii*, *Golenkinia radiata*, *Syracosphaera pirus*, *Corethron criophilum*, *Cochliopodium vestitum* and Chlamydomonas sp. had a mutualism symbiosis with their symbiotic bacteria. Bacteria that were successfully cultured were divided into 4 species, those were: *Clostridium* sp., *Streptococcus* sp., *Veillonella* sp., and *Staphylococcus* sp. It was a mutualism symbiosis involving cobalamin and thiamine. Cobalamin and thiamine increased in the exponential phase along with the increasing quantity of the colony of *Staphylococcus* sp. The interaction of the

microalgae consortium of Glagah with symbiotic bacteria was able to increase the number of microalgae cells, and also chlorophyll a and b levels. Therefore, the biomass level also increased with the increasing quantity of the cell microalgae and chlorophyll a and b level.

AUTHOR CONTRIBUTION

T.W.S. collected, analysed data, and wrote the manuscript. E.A.S designed the research, wrote, and reviewed the manuscript. A.B. wrote and reviewed the manuscript.

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CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

Amin, S.A. et al., 2015. Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature*, 522, pp.98–101. doi: 10.1038/nature14488.

Cheng, J. et al., 2016. Enhancing the growth rate and astaxanthin yield of Haematococcus pluvialis by nuclear irradiation and high concentration of carbon dioxide stress. *Bioresource Technology*, 204, pp.49–54. doi: 10.1016/j.biortech.2015.12.076

Cheng, Z., Yamamoto, H. & Bauer, C.E., 2016. Cobalamin's (Vitamin B12) Surprising Function as a Photoreceptor. *Trends Biochem. Sci.*, 41(8), pp.647-650. doi: 10.1016/j.tibs.2016.05.002.

Cooper, M.B. & Smith, A.G., 2015. Exploring mutualistic interactions between microalgae and bacteria in the omics age. *Curr. Opin. Plant Biol.*, 26, pp.147–153. doi: 10.1016/j.pbi.2015.07.003.

Croft, M.T., Warren, M.J. & Smith, A.G., 2006. Algae need their vitamins. *Eukaryot. Cell*, 5, pp.1175–1183. doi: 10.1128/EC.00097-06.

Foster, R.A. et al., 2011. Nitrogen fixation and transfer in open ocean diatom-cyanobacterial symbioses. *The ISME Journal*, 5, pp.1484–1493. doi: 10.1038/ismej.2011.26.

Grenni, P., Ancona, V. & Barra Caracciolo, A., 2018. Ecological effects of antibiotics on natural ecosystems: A review. *Microchem. J.*, 136, pp.25–39. doi: 10.1016/j.microc.2017.02.006.

Jiang, L. et al., 2007. Quantitative studies on phosphorus transference occuring between *Microcystis aeruginosa* and its attached bacterium (*Pseudomonas* sp.). *Hydrobiologia*, 581, pp.161–165. doi: 10.1007/s10750-006-0518-0.

Kazamia, E. et al., 2012. Mutualistic interactions between vitamin B12-dependent algae and heterotrophic bacteria exhibit regulation. *Environ. Microbiol.*, 14, pp.1466–1476. doi: 10.1111/j.1462-2920.2012.02733.x.

Konopka, A., Lindemann, S. & Fredrickson, J., 2015. Dynamics in microbial communities: Unraveling mechanisms to identify principles. *The ISME Journal*, 9, pp.1488–1495. doi: 10.1038/ismej.2014.251.

Leisico, F. et al., 2018. First insights of peptidoglycan amidation in Gram -positive bacteria - the high-resolution crystal structure of *Staphylococcus aureus* glutamine amidotransferase GatD. *Scientific Reports*, 5, 5313. doi: 10.1038/s41598-018-22986-3.

Li, D. et al., 2011. Bacterial community characteristics under long-term antibiotic selection pressures. *Water Res.*, 45(18), pp.6063–6073. doi: 10.1016/j.watres.2011.09.002.

Matsui, K., Ishii, N. & Kawabata, Z., 2003. Release of extracellular transformable plasmid DNA from Escherichia coli cocultivated with algae. *Appl. Environ. Microbiol.*, 69, pp.2399–2404. doi: 10.1128/AEM.69.4.2399-2404.2003.

Moulin, M. et al., 2013. Analysis of Chlamydomonas thiamin metabolism in vivo reveals riboswitch plasticity. *Proc. Natl. Acad. Sci. U. S. A.*, 110(36), pp.14622–14627. doi: 10.1073/pnas.1307741110.

Müller, I.B. et al., 2009. The Vitamin B1 Metabolism of Staphylococcus aureus Is Controlled at Enzymatic and Transcriptional Levels 4. *PLoS One*, 4(11), e7656. doi: 10.1371/journal.pone.0007656.

Rosnow, J.J. et al., 2018. A cobalamin activity-based probe enables microbial cell growth and finds new cobalamin-protein interactions across domains. *Appl. Environ. Microbiol.*, 84(18), e00955. doi: 10.1128/AEM.00955-18.

Suyono, E.A., Retnaningrum, E. & Ajijah, N., 2018. Bacterial symbionts isolated from mixed microalgae culture of Glagah strains. *Int. J. Agric. Biol.*, 20(1), pp.33–36. doi: 10.17957/IJAB/15.0326.

Voitsekhovskaja, O. V. & Tyutereva, E. V., 2015. Chlorophyll b in angiosperms: Functions in photosynthesis, signaling and ontogenetic regulation. *J. Plant Physiol.*, 189, pp.51–64. doi: 10.1016/j.jplph.2015.09.013.

Wang, H. et al., 2016. Effects of bacterial communities on biofuel-producing microalgae: Stimulation, inhibition and harvesting. *Crit. Rev. Biotechnol*, 36(2), pp.341–352. doi: 10.3109/07388551.2014.961402.

Warren, M.J. et al., 2002. The biosynthesis of adenosylcobalamin (vitamin B12). *Nat. Prod. Rep.*, 19(4), pp.390–412. doi: 10.1039/b108967f.

Whitehead, T.R. et al., 2008. Catabolic pathway for the production of skatole and indoleacetic acid by the acetogen Clostridium drakei, Clostridium scatologenes, and swine manure. *Appl. Environ. Microbiol.*, 74(6), pp.1950–1953. doi: 10.1128/AEM.02458-07.