Understanding of mutualistic interaction between marine phytoplankton (*Tetraselmis striata*) and bacteria (*Pelagibaca bermudensis* and *Stappia* sp.) in phycosphere

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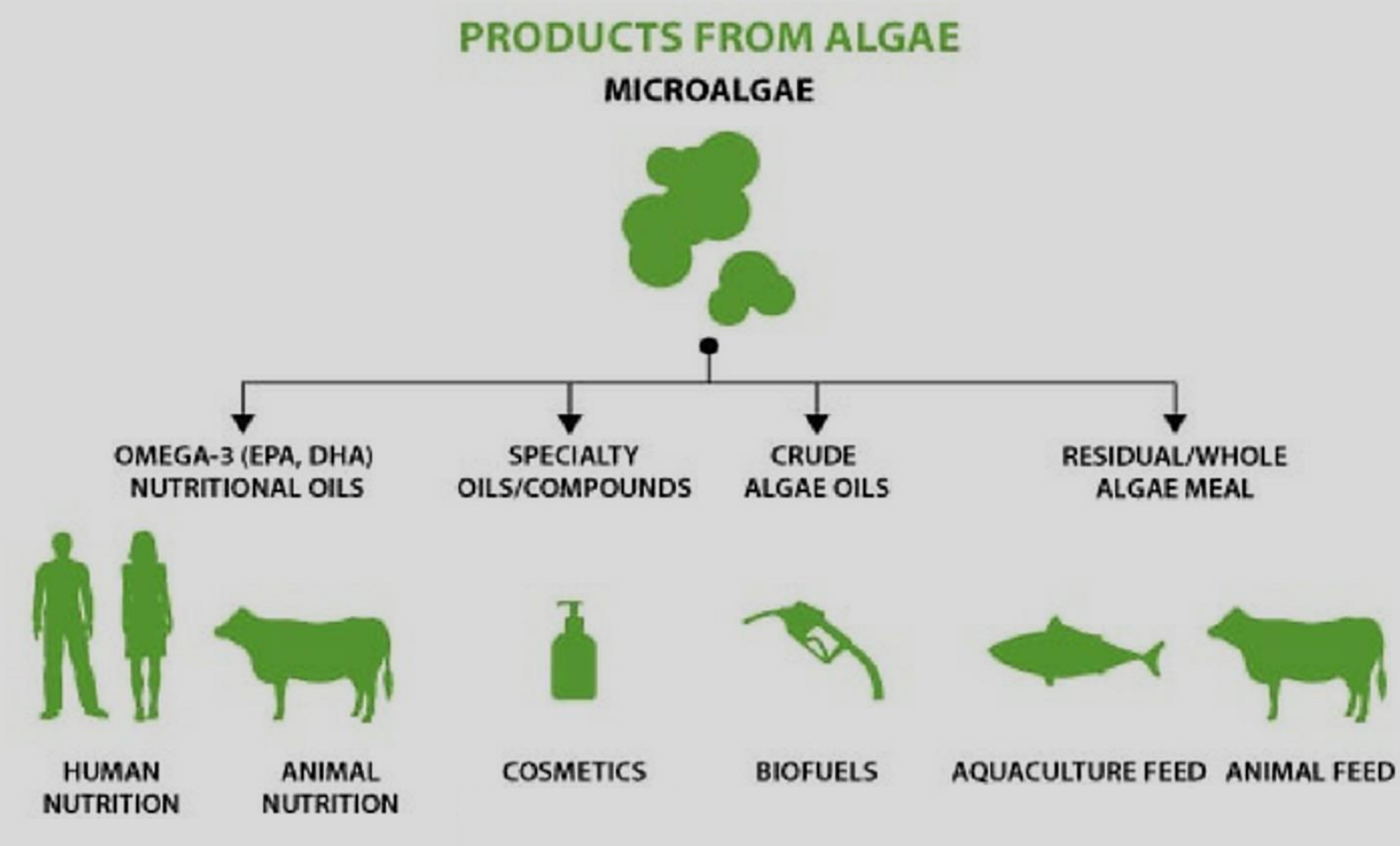
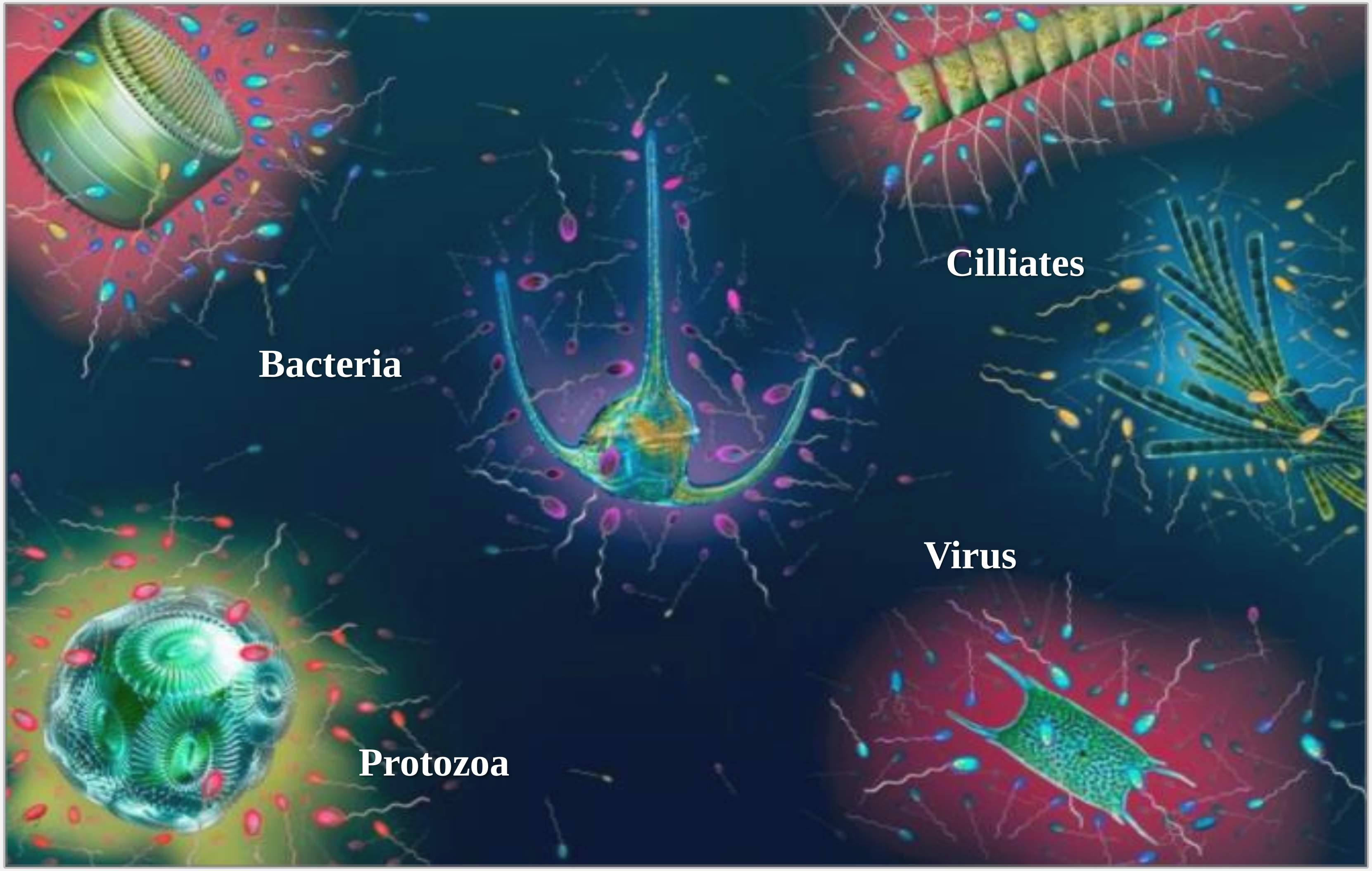
Why?



Reference – AlgencalB

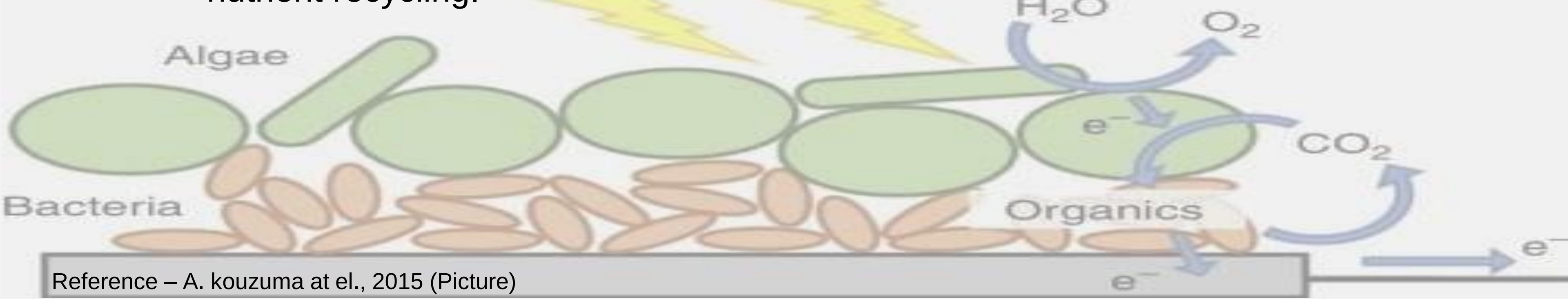
Phycosphere



Reference – microscaleocean.org

Phycosphere

- The phycosphere is a microalgae mucus region that is rich in organic matter surrounding a phytoplankton cell.
- This area is high in nutrients due to extracellular waste from the phytoplankton cell and it has been suggested that bacteria inhabit this area to feed on these nutrients.
- This high nutrient environment creates a microbiome and a diverse food web for microbes such as bacteria and protists.
- It has also been suggested that the bacterial assemblages within the phycosphere are species-specific and can vary depending on different environmental factors.
- In terms of comparison, the phycosphere in phytoplankton as been suggested analogous to the rhizosphere in plants, which is the root zone important for nutrient recycling.

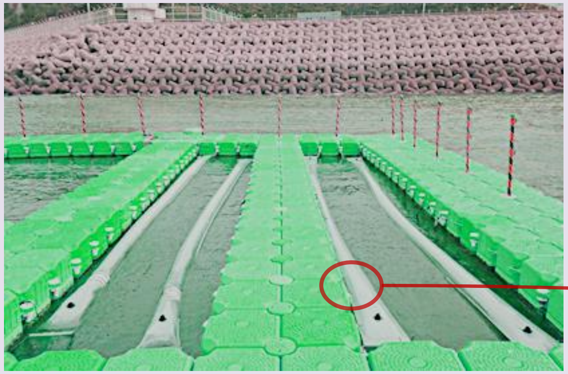


Reference – A. kouzuma at el., 2015 (Picture)

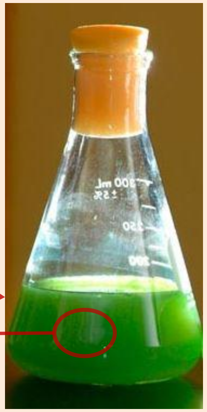
Questions

- First, a specific bacterium have an ability to enhance algal biomass?
- Second, are theses bacterial stimulating effects universal or species-specific?
- Then, what is a plausible mechanism of algal–bacterial growth promoting effects?
- Finally, these bacteria are able to survive in a strong competition against native bacteria even in xenic natural seawater?

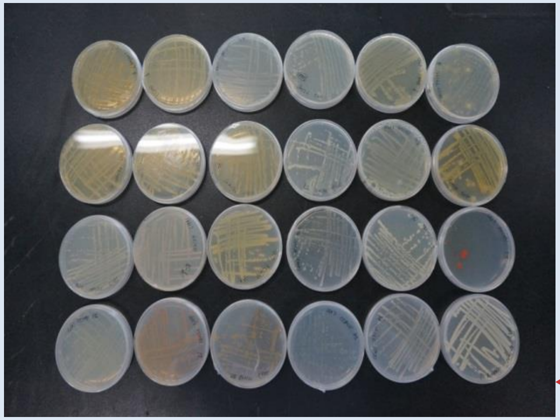
Preparation for Experimental Axenic *T. striata* and Bacterial Isolates



Tetraselmis striata (KCTC 12432BP) was utilized for the experiment from mass cultivation in South Korea.



Initially, to eliminate bacteria associated with *T. striata* and to obtain axenic cultures, antibiotic treatment was applied.



A total of 26 bacterial isolates were obtained from the initial xenic culture of *T. striata.*

T. striata was co-cultured with individual bacterial strains, if any bacterium shows growth promoting effects on microalgae.

Bacteria strain	Growth promoting effects
	Tetraselmis sp. KCTC12432BP
HYYH-1409-2	-0.33
HYYH-1409-3	-0.07
HYYH-1409-4-1	-0.19
HYYH-1409-4-3	1.66
HYYH-1409-7	-0.27
HYYH-1409-8	-0.38
HYYH-1409-10-1	-0.48
HYYH-1409-11-1	-0.42
HYYH-1409-11-2	-0.33
HYYH-1409-12	-0.46
HYYH-1409-13	-0.19
HYYH-1409-16-1	0.00
HYYH-1409-16-2	2.29
HYYH-1409-17-1	2.48
HYYH-1409-17-3	3.04
HYYH-1410-18-1	-0.04
HYYH-1410-18-2	0.00
HYYH-1410-18-3	0.16
HYYH-1410-19	-0.19
HYYH-1410-20	-0.20
HYYH-1410-21	-0.18
HYYH-1410-23	-0.15
HYYH-1410-25	-0.19
HYYH-1410-26	-0.07
HYYH-1410-28	0.76
HYYH-1410-39	1.43

Table 1 | Preliminary screening test for a total of **26 bacterial isolates** from *Tetraselmis* sp. culture. The growth promoting effects were calculated (after 10days) according to Eq. (1) below.

(1) Equation:

$$\text{Growth effect} = \frac{D_{\text{T-Treatment}} - D_{\text{T-Control}}}{D_{\text{T-Control}}}$$

Table. 4. 25 bacterial strains used for screening test on *Tetraselmis sp.* in this study were applied to *Heterosigma akashiwo*, *Chattonella marina*, *Amphidinium* sp. and *Dunaliella teriolecta* respectively. The growth promoting effects were calculated according to Eq. (1) in Section 2.

$$\text{(1) Equation:} \quad \text{Growth effect} = \frac{D_{\text{T-Treatment}} - D_{\text{T-Control}}}{D_{\text{T-Control}}}$$

Bacteria strain	Growth promoting effects			
	Heterosigma akashiwo	*Chattonella marina*	*Amphidinium* sp.	*Dunaliella teriolecta*
HYYH-1409-2	0.21	-0.07	-0.04	-0.43
HYYH-1409-3	0.11	-0.80	0.37	-0.03
HYYH-1409-4-1	0.34	-0.30	0.04	-0.64
HYYH-1409-4-3	**0.21**	**-0.60**	**-0.04**	**-0.67**
HYYH-1409-7	0.74	-0.83	0.26	-0.16
HYYH-1409-8	0.32	-0.87	-0.11	-0.01
HYYH-1409-10-1	-0.98	-0.97	-0.83	0.10
HYYH-1409-11-1	0.42	-0.90	-0.41	-0.07
HYYH-1409-11-2	1.63	-0.90	-0.02	-0.27
HYYH-1409-12	0.34	-0.87	0.02	-0.28
HYYH-1409-13	0.05	-0.60	-0.04	-0.46
HYYH-1409-16-2	0.74	-0.83	0.26	-0.16
HYYH-1409-17-1	0.37	-0.90	-0.37	-0.73
HYYH-1409-17-3	**1.11**	**-0.63**	**-0.07**	**-0.76**
HYYH-1409-18-2	1.53	-0.83	0.30	-0.43
HYYH-1409-18-3	1.21	-0.73	0.23	-0.58
HYYH-1409-19	-0.05	-0.97	-0.17	-0.40
HYYH-1409-20	0.26	-0.73	0.04	-0.25
HYYH-1409-21	0.68	-0.83	0.04	-0.09
HYYH-1409-23	-0.26	-0.97	0.00	-0.10
HYYH-1409-25	-0.05	-0.97	-0.17	-0.40
HYYH-1409-28	-0.21	-0.93	0.00	-0.27
HYYH-1409-37	0.53	-0.70	0.52	-0.55
HYYH-1409-39	0.37	-0.93	-0.28	-0.76

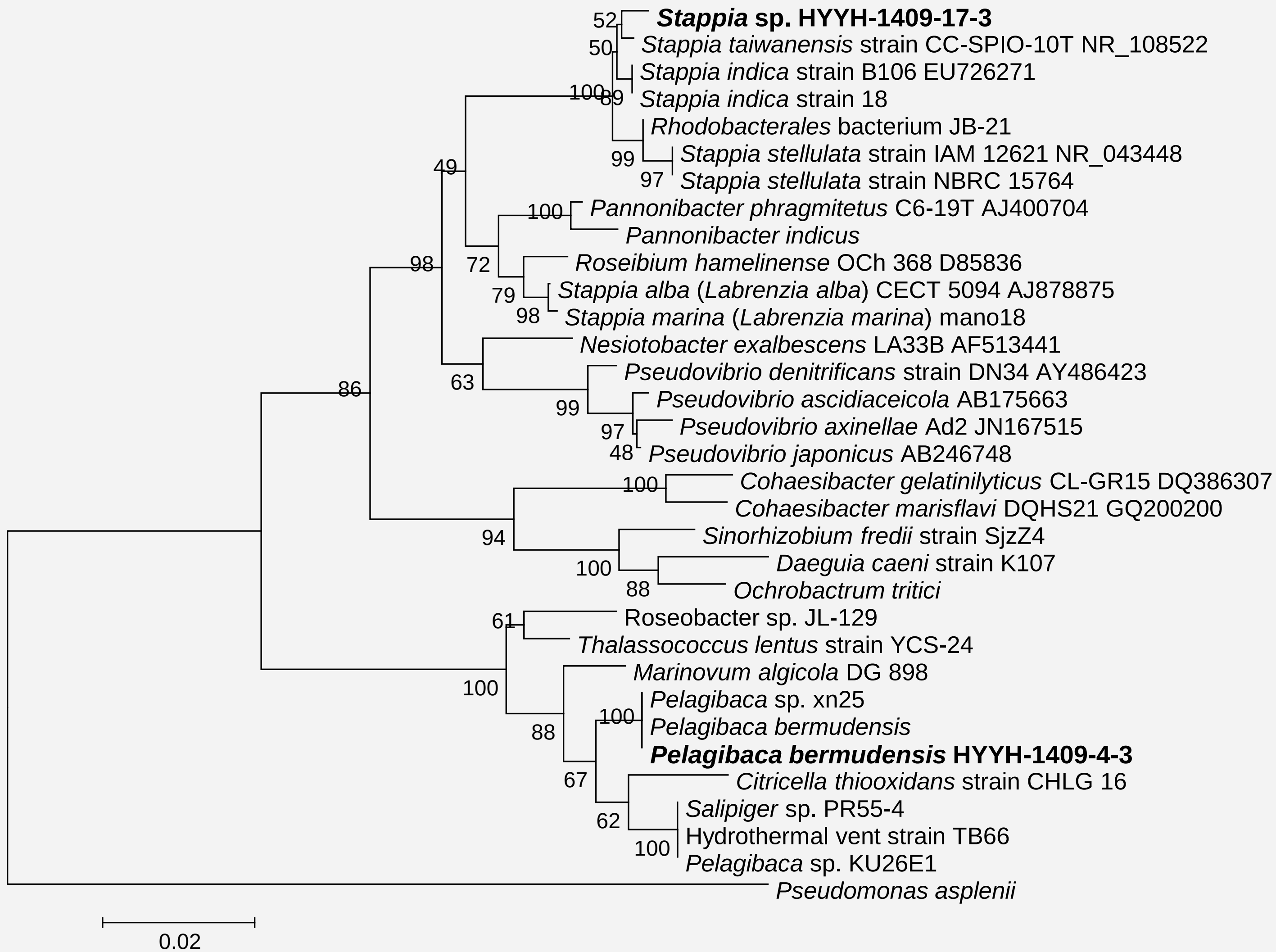


FIGURE 1 | Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.31595249 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 33 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 883 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

Mutualistic relation between Microalgae and Bacteria

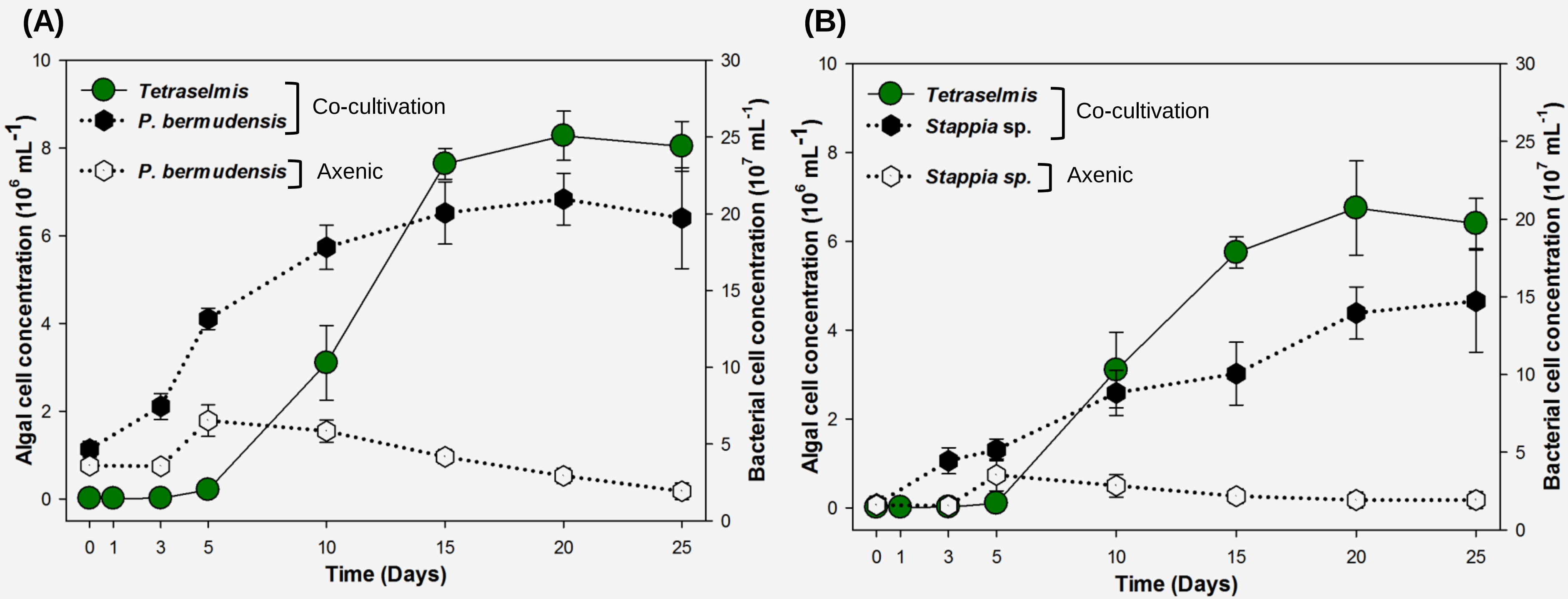
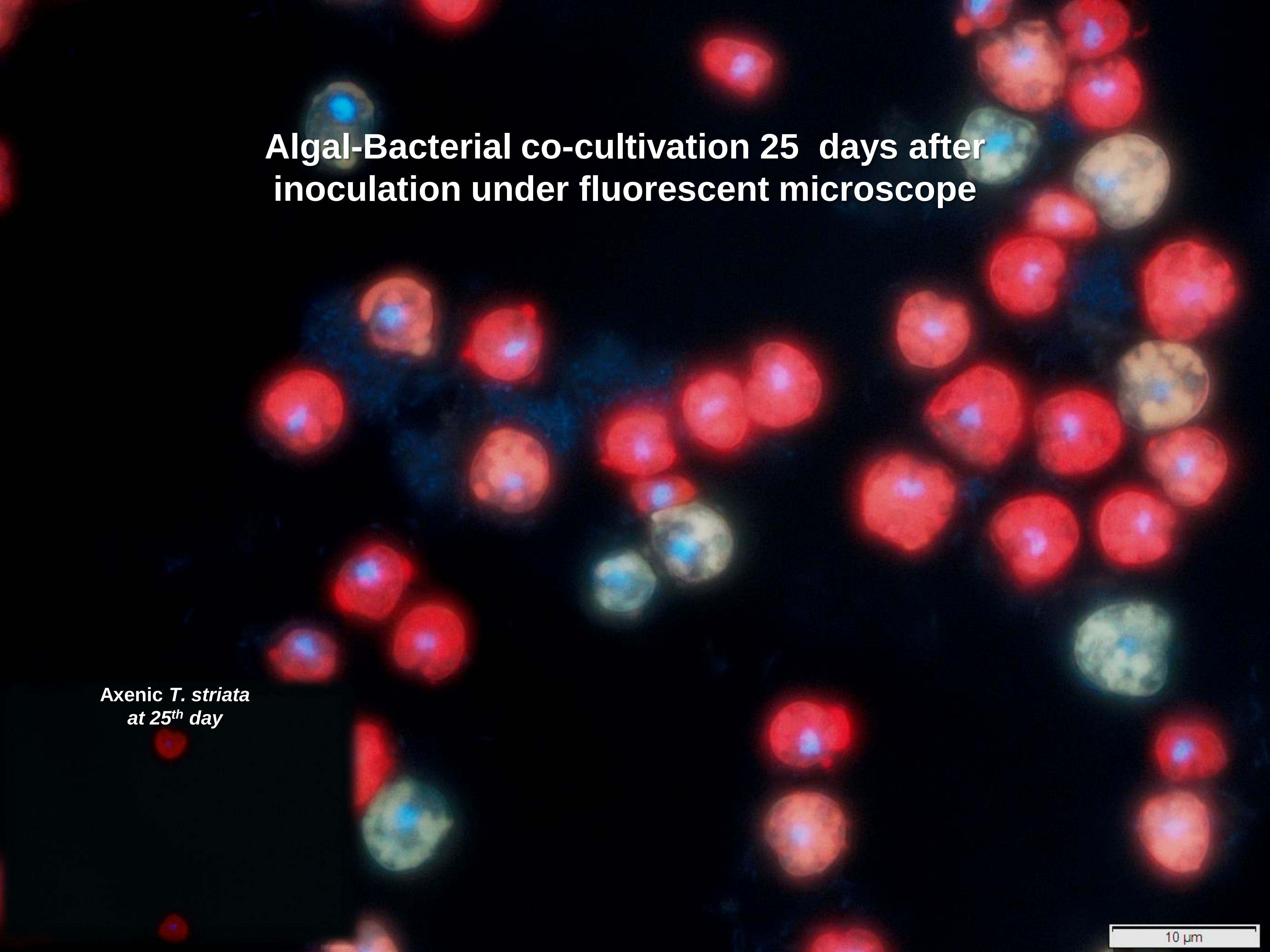


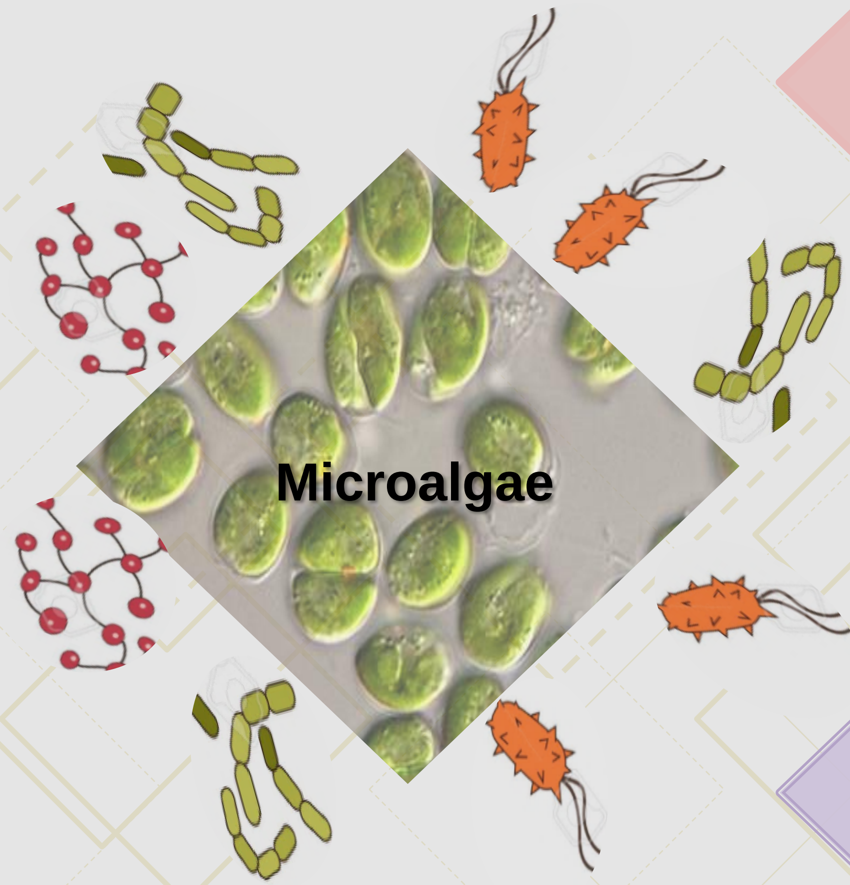
FIGURE 2 | Cell concentration of *T. striata* with bacteria (**A** = *Pelagibaca bermudensis* and **B** = *Stappia* sp.) in co-cultivation condition and axenic bacteria (**A** = *P. bermudensis* and **B** = *Stappia* sp.) during the growth in O3 media.


Algal-Bacterial co-cultivation 25 days after inoculation under fluorescent microscope
Axenic *T. striata* at 25th day
10 µm

Possible Benefits from Phycospheric Bacteria

Phytohormones
Ex) AAI and TDA…

Unknown metabolites



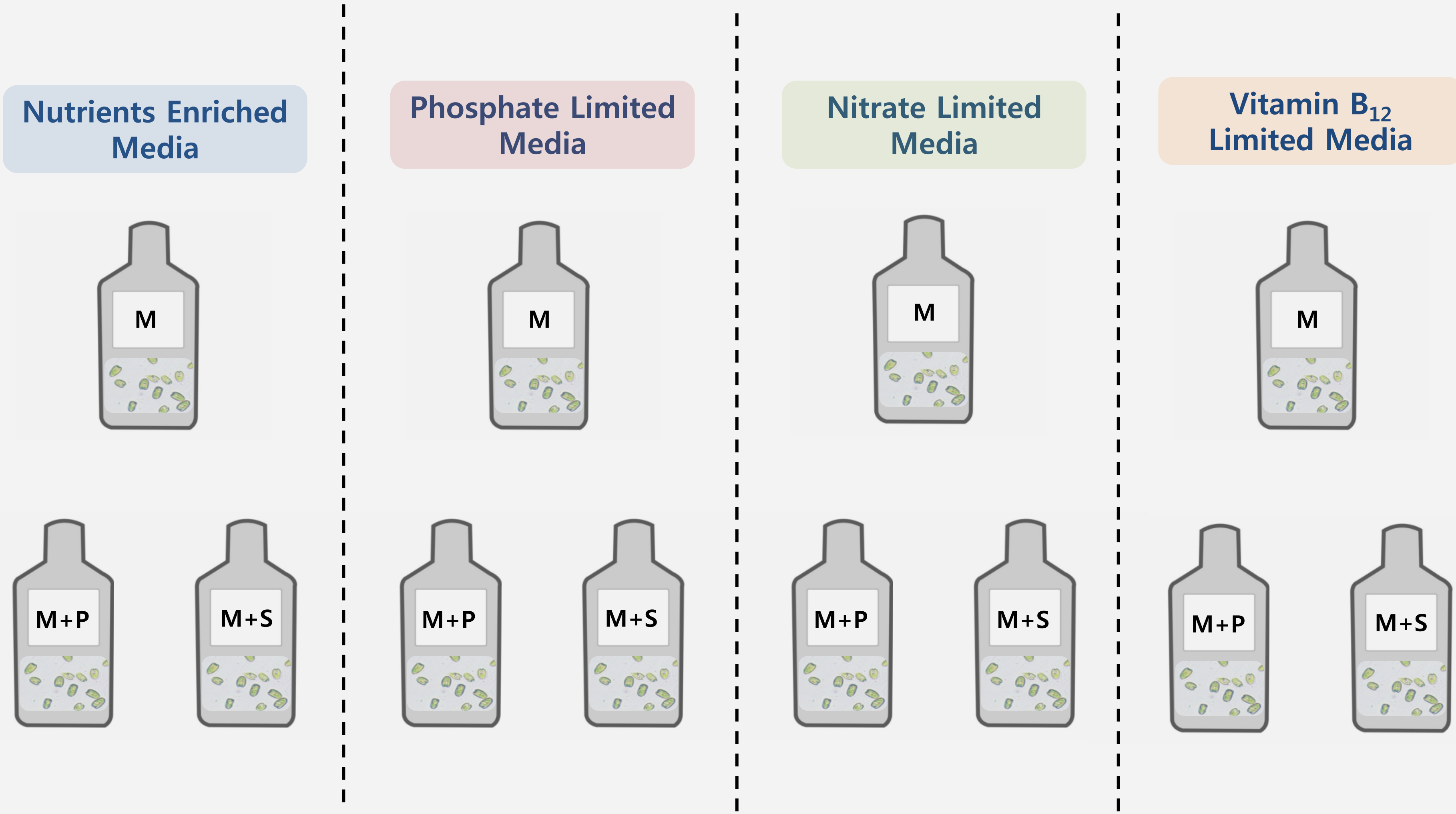
Inorganic Nutrients
Ex) N, P and Vitamin..

Excess oxygen↓
and
Carbon dioxide↑

Nutrients related mechanisms of bacteria in phycsophere

M – Microalgae (Tetraselmis striata)

P – Bacteria (*Pelagibaca bermuensis*)
S – Bacteria (*Stappia* sp.)



150ml volume

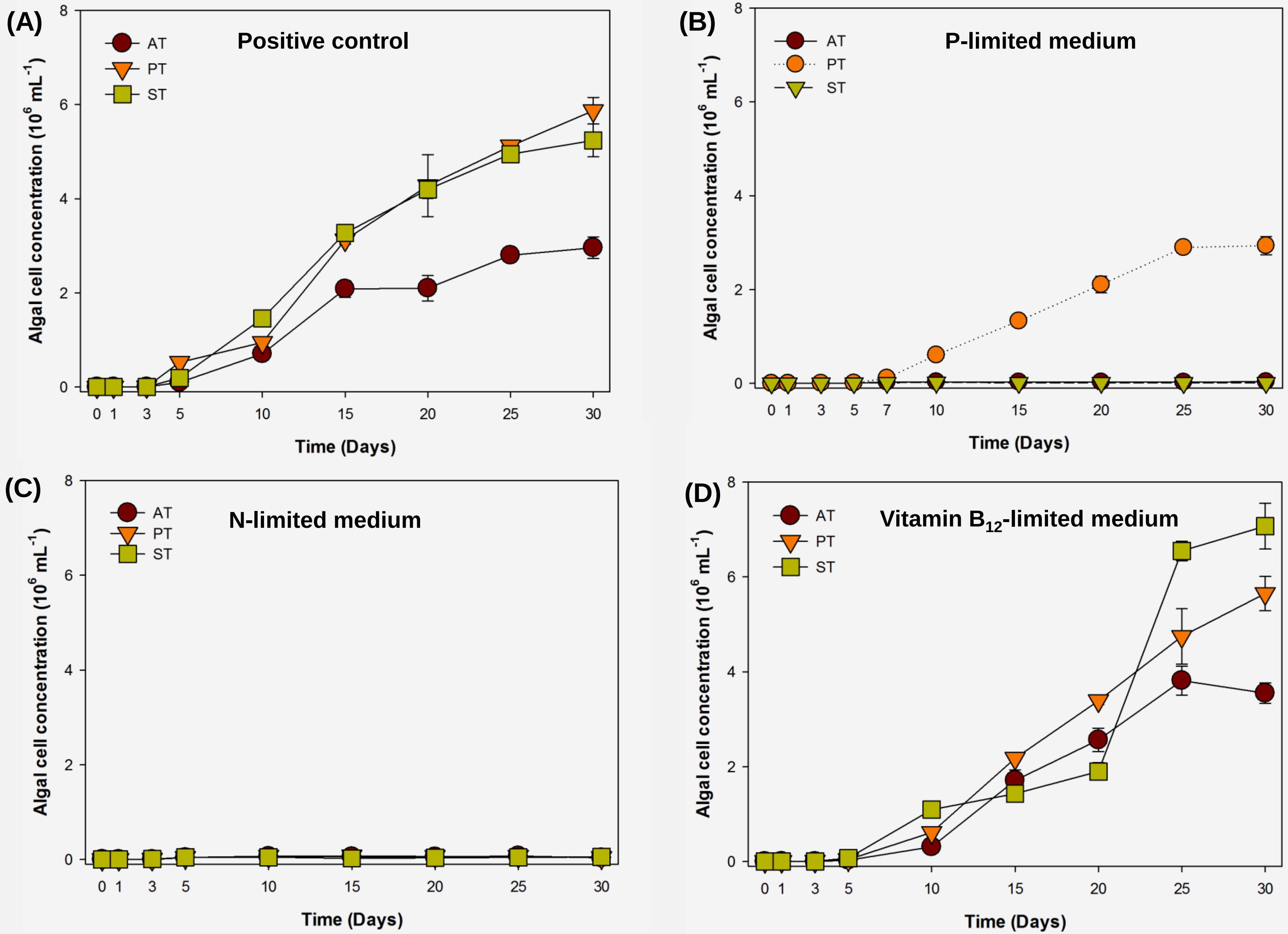
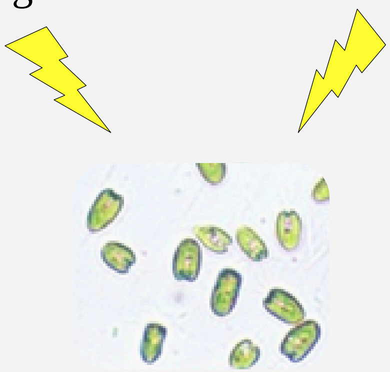


FIGURE 3 | Growth rates of *T. striata* (KCTC12432BP) in varied cultivation system under **(A)** enriched nutrients conditions, **(B)** phosphate-limited conditions, **(C)** nitrate-limited conditions, and **(D)** vitamin B_{12} limited conditions, respectively (AT = Axenic *T. striata*, PT = Mixed culture of *P. bermudensis* and *T. striata*, ST = Mixed culture of *Stappia* and *T. striata*).

Designing the Dead Algal Cell Experiment

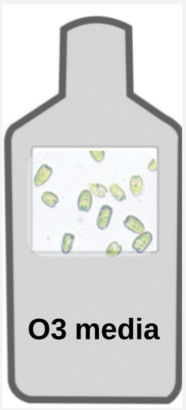
Liquid nitrogen freezing

UV



Preserved at a deep freezer (-70˚C)

Inoculation



Dead algal cell confirmation

	Day	Cell abundance
	0	42,500 cells /mL^{-1}
	10	34,800 cells /mL^{-1}
Dead!	20	29,100 cells /mL^{-1}

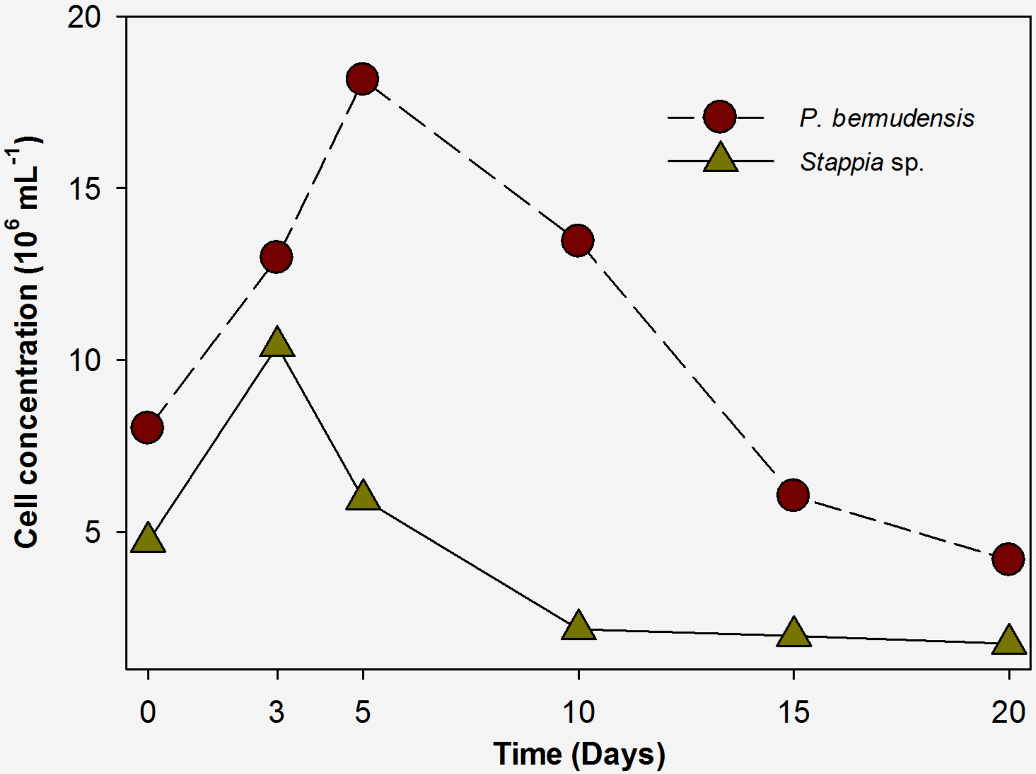


FIGURE 4 | Bacterial abundance (*P. bermudensis* and *Stappia* species) with organic source from metabolically inactive *T. striata*.

	Axenic *T. striata* (Control)			***P. bermudensis* – *T. striata***			***Stappia* sp. – *T. striata***		
	TP	DIP	DOP	TP	DIP	DOP	TP	DIP	DOP
0 Day	0.57 ± 0.01	ND	ND	22.91 ± 0.01	**ND**	**ND**	9.09 ± 0.1	ND	ND
5 Day	0.50 ± 0.02	ND	ND	22.13 ± 0.02	**5.13 ± 0.1**	**3.31 ± 0.1**	8.56 ± 0.02	ND	ND
15 Day	0.47 ± 0.01	ND	ND	22.02 ± 0.01	**6.12 ± 0.1**	**0.80 ± 0.2**	9.19 ± 0.2	ND	ND
20 Day	0.63 ± 0.01	0.13 ± 0.08	ND	22.88 ± 0.01	**12.76 ± 0.1**	**1.42 ± 0.4**	9.40 ± 0.6	0.23 ± 0.03	ND

*(ND, no detection; TP, Dissolved + Total particulate phosphate; DIP, dissolved inorganic phosphate; and DOP, dissolved organic phosphate).

TABLE 2 | Phosphate concentration (µM) in the metabolically inactive *Tetraselmis striata* containing cultures inoculated together with growth promoting bacteria in phosphate-limited O3 media (all phosphate measurement on 0 day was carried out after inoculation of cells).

Algal Biomass

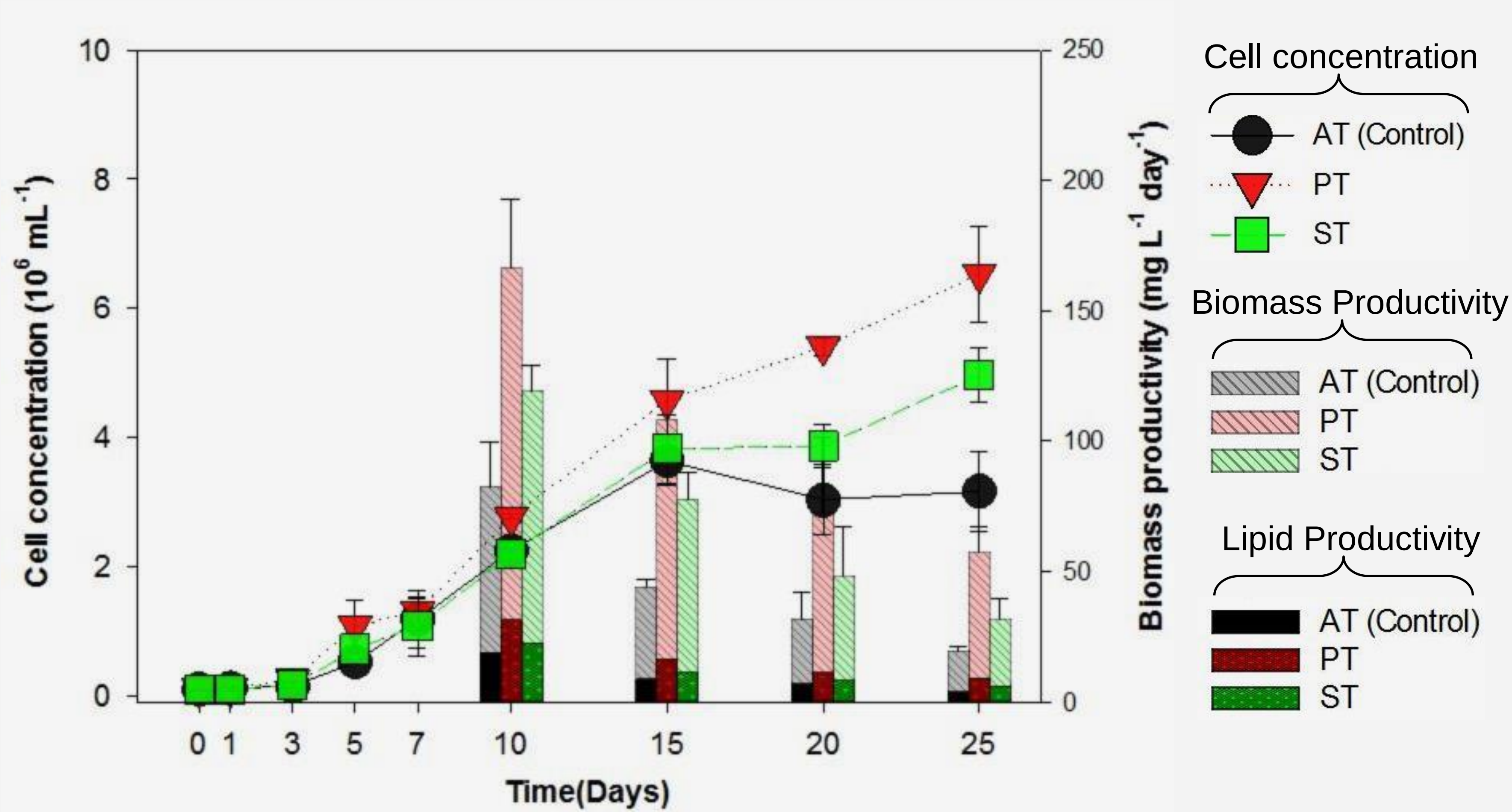


FIGURE 5 | Cell concentration, biomass productivity (mg L^{-1} day^{-1}) and lipid productivity (mg L^{-1} day^{-1}) of *Tetraselmis striata* with co-cultivation of TGPB (AT = Axenic *T. striata*, PT = Mixed culture of *Pelagibaca bermudensis* and *T. striata*, ST = Mixed culture of *Stappia* and *T. striata*).

Analysis of Lipid Contents in Microalgal Biomass

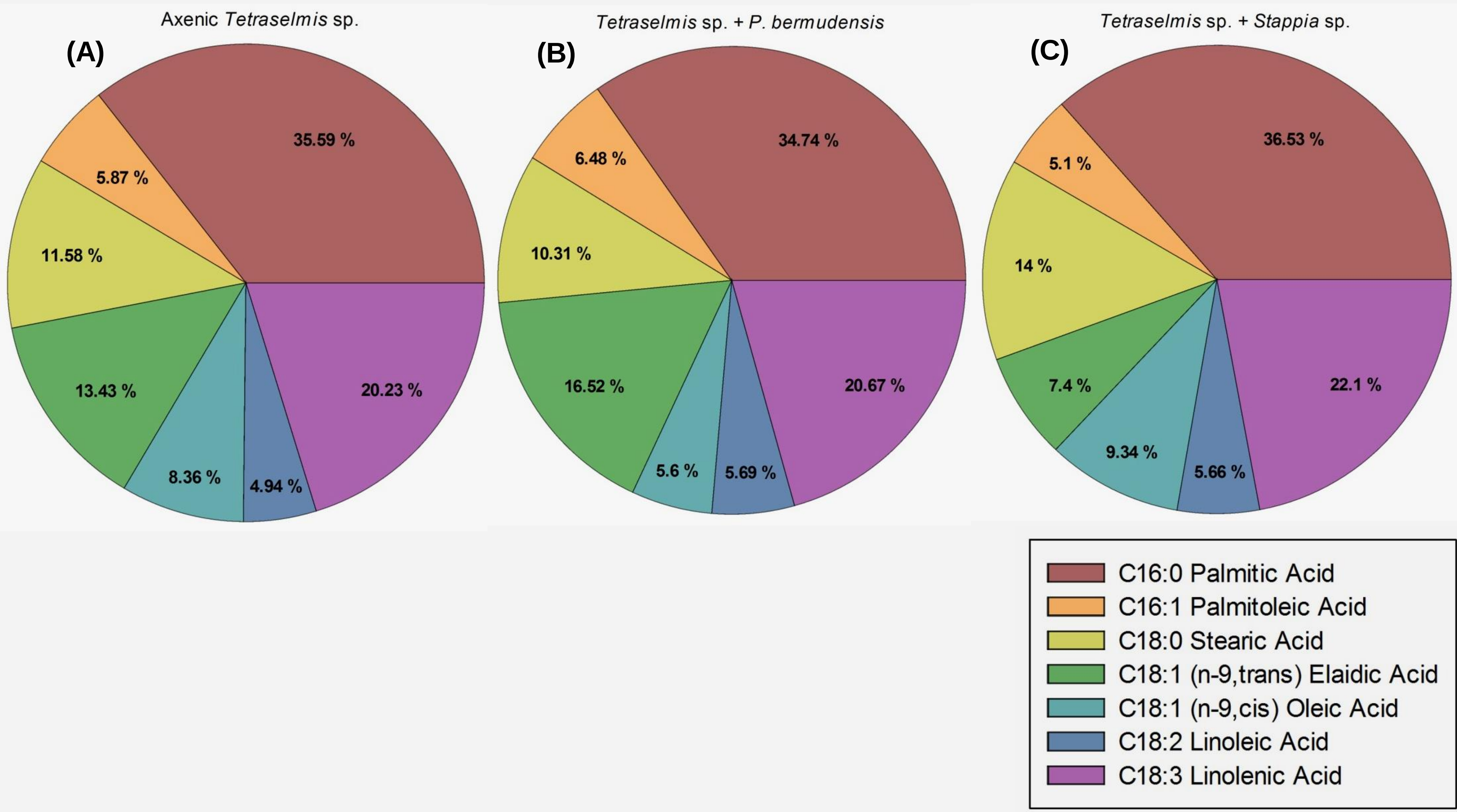
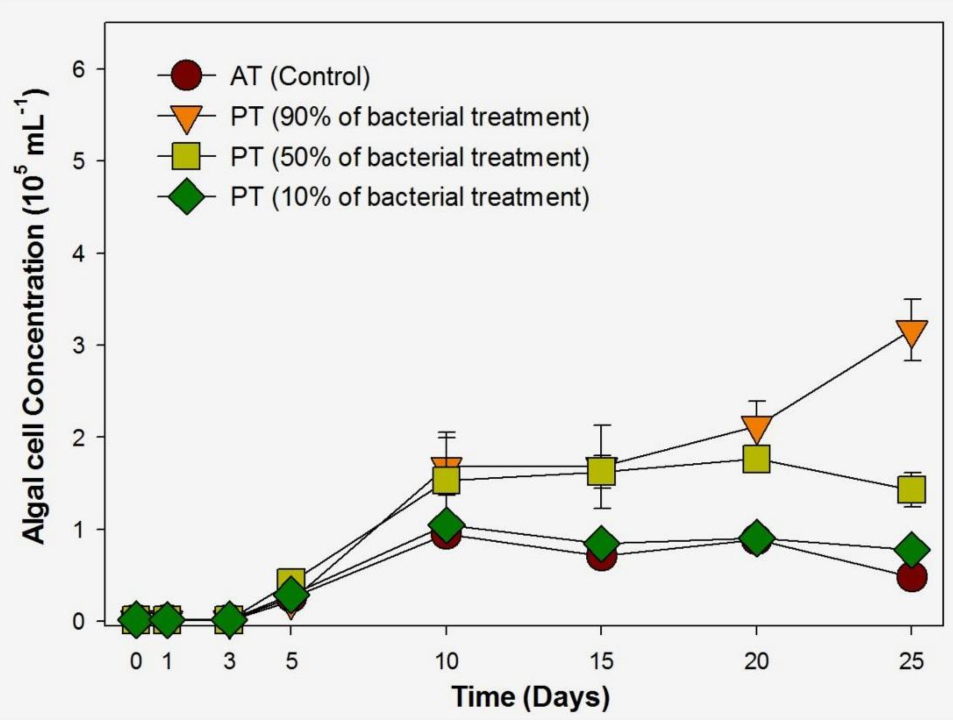


FIGURE 6 | Fatty Acid Methyl Ester (FAME) profiles in cultures of microalgae: (A) axenic condition, (B) inoculated *P. bermudensis*, and (C) inoculated *Stappia* sp. respectively. (30th day)

Algal Growth Rates With Different Bacterial Concentrations

(A)



(B)

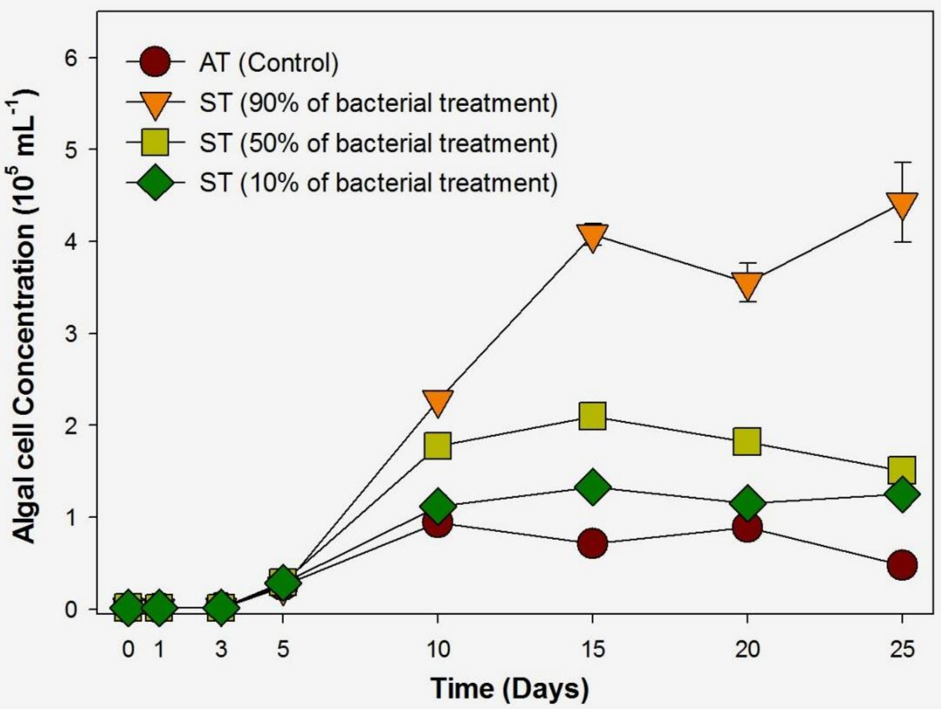


FIGURE 7 | Growth rates of *T. striata* (KCTC12432BP) in xenic seawater collected from Youngheng, South Korea. The percentage (90, 50, and 10%) indicates initial inoculating density of **(A)** *P. bermudensis* and **(B)** *Stappia* species against native bacterial diversity in nature (AT = Axenic *T. striata*, PT = Mixed culture of *P. bermudensis* and *T. striata*, ST = Mixed culture of *Stappia* and *T. striata*).

Changes in Bacterial Compositions

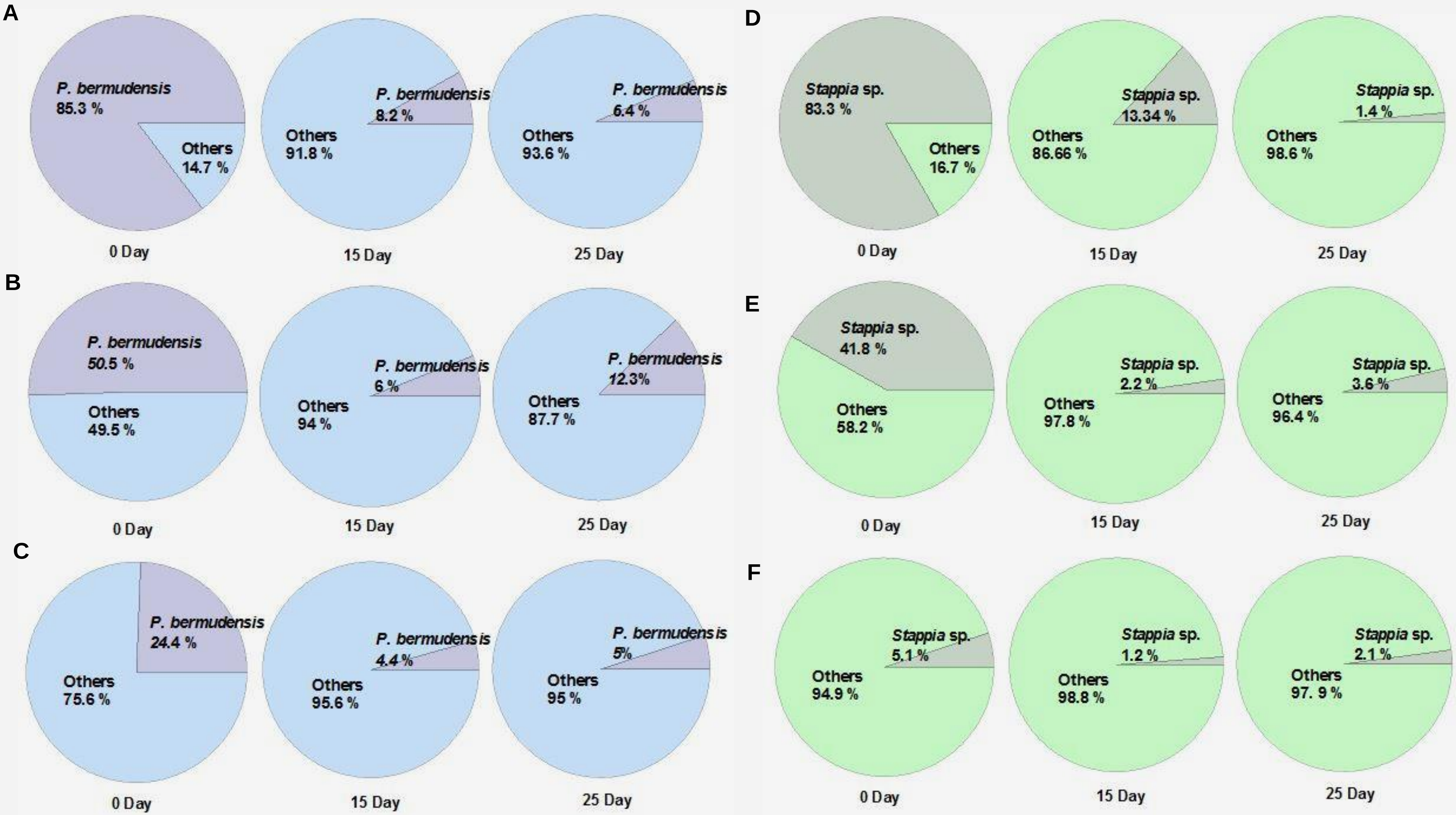


FIGURE 8 | Changes of the occupancy ratio of TGPB in xenic seawater (0.2 ㎛ filtered) co-cultivated with *T. striata* during the growth (**A** = 85.3% of *P. bermudensis* initial inoculation; **B** = 50.5% of *P. bermudensis* initial inoculation; **C** = 24.4% of *P. bermudensis* initial inoculation; **D** = 83.3% of *Stappia* sp. initial inoculation; **E** = 41.8% of *Stappia* initial inoculation; **F** = 5.1% of *Stappia* sp. initial inoculation).

Summary

- ✓ Two bacteria (*P. bermudensis* and *Stappia* sp.) were successfully isolated from phycosphere of *T. striata* and selected on account of their significant algal growth promoting effects.
- ✓ Not only *T. striata* reaps benefit from co-cultivation, but is necessary for bacterial survival and growth in this case of aquatic system, thus, it is suggested that they have mutualistic interaction.
- ✓ Particularly, it is plausible that *P. bermudensis* has a function to donate inorganic phosphate to *T. striata* through phycospheric interaction.
- ✓ Algal-bacterial interaction in phycosphere would be a promising breakthrough for better algal cultivation and biomass.

Thank you

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