Exploration of IAA Producing Bacteria And Amylolytic Bacteria From Several East Java Lakes, and Their Potency For Microbial Consortium To Accelerate *Chlorella Vulgaris* Growth

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Abstract

The consortium of various types of bacteria from lakes in East Java has the potency to stimulate microalgae *Chlorella vulgaris* growth. Increased microalgae density from co-culture has an excellent potency for sources of biomass that can be developed for renewable energy. Several stages conducted of this research started from an exploration of IAA producing bacteria and amylolytic bacteria from several East Java Lakes; then, the highest bacterial isolates were identified with morphological and genotypical characteristics. The well-characterized bacterial isolates were used for the microbial consortium in co-culture with *C. vulgaris*. The treatment used in this study as follows: I) *C. vulgaris* without bacteria culture as a control, II) amylolytic bacteria + *C. vulgaris*, III) IAA-producing bacteria + *C. vulgaris*, IV) potential amylolytic bacteria and IAA-producing bacteria + *C. vulgaris*. The exploration result of potential bacteria from Ranu Pani, Ranu Regulo, Telaga Ngebel, and Ranu Grati lakes was found 53 amylolytic bacterial isolates, and 90 isolates IAA-producing bacteria. The highest amylolytic bacteria (isolate L) is related to *Bacillus amyloliquefaciens*, while the most elevated IAA-producing bacteria (isolate C) is related to *Bacillus paramycoides*. The highest cell density was produced in treatment III, reaching 2.7×10^6 cells/mL on day 50th. The treatments with supplement bacteria showed a significant effect for accelerating the growth of microalgae compared to control.

Introduction

Energy dependence and continuous energy consumption could cause an energy crisis. Global demand for petroleum is expected to increase by 40% in 2025, so environmentally friendly alternative fuels are needed (Hirsch et al., 2005). Biofuel from biological sources (biomass) has a potential resource for renewable energy playing an essential role in the future global energy infrastructure (Cheah et al., 2020; Tandon et al., 2017). Biomass from microalgae has been considered one of the most promising raw materials as a renewable energy source because not producing emissions in their process to generate biofuels (Cheah et al., 2020; Chisti, 2007; Tandon et al., 2017). The recent studies with culture *C. vulgaris* showed the advantages of biomass production compared with plants (Tandon et al., 2017). Besides, *C. vulgaris* has rapid growth correlated with numerous lipid production, low cost, and does not consume large scale tracts of land (Chisti, 2007; Tandon et al., 2017).

The problem in the monoculture of microalgae is how the high biomass with low cost and a short period can be obtained. Single-species cultivation for microalgae is challenging for biomass production on a large scale because it is contrary to the natural tendency of an ecosystem to become increasingly complex (Kazamia et al., 2012). Hence, the use of a microbial consortium consisting of several kinds of bacteria and microalgae can optimize the growth of microalgae in co-culture (Kazamia et al., 2012). This microbial consortium with various types of bacteria from lakes in East Java has a great potential to optimize culture condition; this condition is essential for large-scale cultivation. Co-culture between microalgae and bacteria can increase the microalgae growth because those bacteria are providing essential inorganic mineral elements for microalgae (Marañón et al., 2005). The blooms of algae and aquatic plants are inseparable from the influence of decomposing bacteria in the water environment

because decomposing bacteria plays as decomposers of organic components into simpler components as nutrients.

Ranu Pani, Ranu Regulo, Ranu Grati, and Telaga Ngebel are favourite lakes in East Java for vacation. Ranu Pani and Ranu Regulo have an average organic substrate content of water, about 18.18% and 26.06%, respectively (Gazali et al., 2014). Ranu Grati has a pH of 7.67 with average temperatures of 29.07°C and a fertility value of 52.41. Based on the TSI value, Ranu Grati classified it as freshwater that had experienced mild eutrophication. Organic materials in the four lakes come from microalgae, plant parts, micro-tricks, agriculture, and waste. The high content of dissolved organic matter (DOM) is an indicator of the abundance and richness of microbes (Gazali et al., 2014).

Amylolytic bacteria can stimulate the microalgae growth by producing amylase enzymes to break down amylum into simple sugars, like glucose, through several stages (Gazali et al., 2014; Silaban et al., 2020). Those simpler molecules can be directly used for supporting microalgae growth. Amylase enzymes from bacteria are widely used in various industries because the strain has rapid growth and require a short amount of time to produce amylases (Keeney & College, 2007). In general, those enzymes were used in textile industries, food industries, and pulp industries. However, those enzymes can also be used in microalgae culture because this type of bacteria can degrade substrates efficiently then be utilized by algae for its growth (Keeney & College, 2007; Souza & Magalhães, 2010).

On the other hand, bacteria producing indole-3-Acetic Acid (IAA hormone) can provide growth factors for responses to light stimulation in plants, including microalgae (Zhao, 2010). IAA is the exogenous auxin class produced by bacteria through the dependent pathway with the addition of tryptophan and independent pathway without tryptophan (Nonhebel, 2015). Most bacteria produce IAA with dependent pathways, notably in the indole-3-acetamide (IAM) pathway and the indole-3-pyruvate (IPA) pathway (Ozdal et al., 2017). IAA hormone is essential for the growth of microalgae, especially *C. vulgaris* (L. De-Bashan, 2008) due to that hormone regulates the growth process and induces the production of metabolites. The utilization of a controlled consortium of bacteria and microalgae could increase microalgae biomass production (Chisti, 2007; Croft et al., 2005; L. E. De-Bashan et al., 2008; Fuentes, Garbayo, Cuaresma, Montero, González-Del-Valle, et al., 2016). This study aims to determine the effect of the amylolytic bacteria and IAA-producing bacteria on the growth of *C. vulgaris* microalgae in co-culture.

Materials And Methods

Samples collection

Water samples were collected from several lakes in East Java, Indonesia (listed in Table 1) with five stations from each lake. Approximately 1000 mL water samples were stored in a sterile bottle; then, placed into the ice-box to protect the pieces from degradation until the laboratory works.

Table 1
List of sampling location.

No	Lakes	Location	Altitude
1	Ranu Pani	Lumajang regency	2.114 masl
2	Ranu Regulo	Lumajang regency	2.100 masl
3	Ranu Grati	Pasuruan regency	100 masl
4	Telaga Ngebel	Ponorogo	745 masl

Abiotic factors were also measured in this study. Those factors include light intensity using Lux meter SANFIX (LX-1330 B), water temperature, and pH were measured using Refractometer ATAGO S-28E, dissolved oxygen (DO) using oxygen meter (Lutron DO-5510), pH meter Lutron 5509, respectively. Water turbidity was measured using a turbidity meter (TOA-TB 25A).

Isolation of IAA producing bacteria and quantification IAA contents

Single colonies that grew with different characteristics were moved into a new plate that contains TSA media with additional tryptophan (25:1). The positive bacterial isolates to produce IAA were tested qualitatively by the colourimetric method using the Salkowski reagent. This reagent was made by 1 mL of 0.5 M FeCl_3 (1.35 g / 10 mL) and 50 mL of 50% HClO_4 (25 mL HClO_4 + 25 mL H_2O) then the reagent was stored in an untranslucent bottle at room temperature. Single isolates presenting a pink colour after dropped Salkowski reagent indicated they have the activity to produce IAA. Those positive isolates were used for quantitative tests using UV-vis spectrophotometry.

Single isolates from TSA media were cultured on *Tryptic Soy Broth* (TSB) media with an additional tryptophan (200 ppm) with a ratio (25:1). The spectrophotometric method was used for quantifying IAA contents produced by the isolates. The concentration of IAA was analyzed based on the absorbance value absorbed by UV-Visible spectrophotometer compared to the IAA standard curve. The absorbance value was directly proportional to the IAA standard curve and IAA produced by the isolates. The standard curve was made using pure IAA. The wavelength used was 530 nm; this wavelength was chosen based on the colour produced by the interaction between the Salkowski reagent and the IAA (Glickmann & Dessaux, 1995).

Amylolytic bacteria selection and semiquantitative assay of amylolytic bacteria activity

The growth medium was used for the single colonies selection is peptone media 0.1% and Plate Count Agar (PCA) media (instant PCA powder 23.5g / L from Merck, USA). The bacteria from water samples were cultured using the serial dilution method with 0.1% peptone buffer then spread into PCA media. Colonies that grew in PCA media were selected based on different colony morphologies to get the single isolates. NA-modified media that was composed of instant nutrient agar (NA) powder 20g / L (Merck, USA) with an additional 2g of amylum (Sigma-Aldrich, USA) was used for selecting and calculating the hydrolysis index from amylolytic bacteria. These bacterial isolates will produce a clear zone around the

colony after dropped the iodine. The bacterium sample was aseptically inoculated into modified-NA media using the quadrant method. The amylum hydrolysis index of each isolate can be determined based on the diameter of the clear zone formed on the amylum agar medium.

The formula for calculation the amylum hydrolysis index is as follows:

$$\text{IHA} = \frac{(\text{d of bacterial colony} + \text{d of clear zone}) - \text{d of bacterial colony}}{\text{d of bacterial colony}}$$

d = Diameter

Amylum hydrolysis index data obtained were then analyzed using ANOVA.

Modified-Gusrina Media preparation

The Gusrina media that has been modified was made by 45 ppm urea, 30 ppm TSP (Triple Super Phosphate), amount of 1 ppm FeCl₃, and 25 µg / L of vitamin B12 (Hadiyanto et al., 2012). Those components dissolved with distilled water up to 1000 mL into an Erlenmeyer flask then heated with a hot plate. This media stock was made with 10× concentration then it can be stored in the refrigerator.

Bacterial stock culture and algae stock culture for microbial consortium

The culture of the highest amylolytic bacteria and the highest ability of IAA-producing bacteria were cultured in 15 mL of nutrient broth (NB) medium at 20°C for 24 h. These bacteria were measured the cell density using the 0.5 Mac. Farland solution scale that has equivalent to 1.5 × 10⁸cfu/mL. These bacterial cultures conditions were used as a starter in co-culture.

The microalgae strain that was used in this study is *C. vulgaris* (Purchased from LIPI, Indonesia). The stock of *C. vulgaris* was made with a ratio of 1:4 between the cultures and the medium. The 2,000 mL of the *C. vulgaris* stocks, which have optimal growth, were moved into a plastic tank with capacity up to 15,000 mL, then 10 mL of the Gusrina medium and distilled water up to 10,000 mL were added. After in exponential phase, 1,000 mL of stocks were divided into 20 tank glasses. These tank glasses were used for the consortium in co-culture *C. vulgaris*.

Consortium condition in microbial consortium with co-culture *C. vulgaris*

The medium used for co-culture *C. vulgaris* contains 0.5 mL of Gusrina modified media, 50 mL stock of microalgae, and 400 mL of sterile water. Those solutions for the co-culture medium were placed into a closed-tank glass that has a capacity of 1,000 mL. The treatments were used in co-culture can be seen in Table 2.

Table 2
The treatments used in this study.

Treatments	Composition
I (control)	50 mL <i>C. vulgaris</i> without the bacteria
II	50 mL <i>C. vulgaris</i> + 50 mL amylolytic bacteria
III	50 mL <i>C. Vulgaris</i> + 50 mL IAA-producing bacteria
IV	50 mL <i>C. vulgaris</i> + 25 mL amylolytic bacteria + 25 mL IAA-producing bacteria

The co-culture was given an aerator set (12 h aerator: 12 h without aerator) and lighting time (16 h lighting: 8 h without light exposure) (Hadiyanto et al., 2012; Lirofiatillah et al., 2020). Those treatments were repeated five times then placed in a closed room at a light intensity of $100\text{--}200\ \mu\text{E sec}^{-1}\text{ m}^{-2}$, 25°C .

Chlorella vulgaris cells density calculation and data analysis

Calculation of *C. vulgaris* cells density was using a hemocytometer. The observation was conducted every two days until the 50th days. The bacterial growth was observed every three days. The 50 μL sample was taken from the tank; then, using a dilution technique, the samples were diluted to reach a dilution level of 10^{-6} . At each dilution of 10^{-4} , 10^{-5} , and 10^{-6} , was taken 100 μL , then put into a petri dish containing PCA media, incubated for 24 h at 20°C . The collected data were presented in a graphic, and they were analyzed with one-way ANOVA. A 5% LSD followed the significance test to determine the best treatment.

Results

Environmental conditions from Lakes

The measured abiotic factors from each lake and station were already listed in Table 3.

Table 3
Abiotic factors measured from each lake

Location	Stations	Abiotic factors			
		DO (mg/mL)	pH	Salinity	Water Brightness (cm)
Ranu Regulo	1	8.9	9.38	0.3	75
	2	8.0	8.65	0.2	75
	3	8.4	8.65	0.2	80
	4	11.3	9.3	0	75
	5	11.6	9.4	0.2	75
Ranu Pani	1	4.2	7.24	0.4	80
	2	5.0	6.83	0.3	82
	3	6.0	6.95	0.2	100
	4	11.6	7.3	0.4	50
	5	6.7	6.88	0.4	50
Ranu Grati	1	13.4	8.7	0.2	163
	2	12.3	8.4	0.1	165
	3	13.5	8.6	0.1	368
	4	13.0	8.71	0	757
	5	13.5	8.84	0	757
Telaga Ngebel	1	2.5	6.98	0.2	676
	2	4.3	7.25	0.1	517
	3	8.5	7.69	0.1	383
	4	6.0	7.17	0.1	450

The total isolates of IAA-producing bacteria and produced IAA content

Ninety isolates of bacteria that positively produce IAA from Ranu Grati, Ranu Pani, Ranu Regulo, and Telaga Ngebel were successfully isolated. As a result, the ten highest bacterial isolates based on UV-Visible spectrophotometer measurements were recorded in Table 4, and complete data were listed in (supplementary files 1). The bacterial isolate C from Ranu Pani was able to produce the highest IAA hormone concentration of 158.11 ppm.

Table 4
Ten highest isolates of IAA-producing bacteria to produce IAA

Isolate	Absorbance	Concentration IAA (ppm)	Source
C	0.653	158.11	Ranu Pani, Regulo
G13	0.453	104.05	Ranu Grati
G28	0.298	62.16	Ranu Grati
G33	0.212	38.92	Ranu Grati
G1	0.210	38.38	Ranu Grati
G18	0.176	29.19	Ranu Grati
J	0.168	27.03	Ranu Pani, Regulo
G7	0.161	25.14	Ranu Grati
TN	0.161	25.14	Telaga Ngebel
G4	0.152	22.70	Ranu Grati

The total amylolytic bacteria and their activity

Fifty-three bacterial isolates from Ranu Pani, Ranu Regulo, Ranu Grati, and Telaga Ngebel had been known to be amylolytic. The average results of calculating the most extensive ten amylum hydrolysis index can be seen in Table 5, and full data can be seen in (supplementary files 2). Table 5 shows that the L bacterial isolate from Ranu Pani had the highest hydrolysis index value of 5.89.

Table 5
Ten highest isolates to hydrolyze amylum

Isolates	Hydrolysis index	Souce
L	5.9	Ranu Pani, Regulo
G33	4.7	Ranu Grati
D	4.65	Ranu Pani, Regulo
KN	4.63	Lake of Ngebel
VN	4.15	Lake of Ngebel
IN	4.08	Lake of Ngebel
P	4.02	Ranu Pani, Regulo
G13	3.77	Ranu Grati
V	3.43	Ranu Pani, Regulo
NN	3.12	Lake of Ngebel

Bacterial isolate C and L identification

The morphological characteristics of isolate C and isolate L can be seen in Table 6.

Table 6
Morphological characterization from bacterial isolate C and L

Characteristics	Isolate C	Isolate L
Colony color	White	White
Shape of colony	Circular	Irregular
Colony side	Smooth, gloomy	Choppy, gloomy,
Colony diameter	0.1 cm	0.285 cm
Cell shape	Basil	Basil
Spores	-	Terminal
Gram	+	+

Based on those morphological characteristics, bacterial isolate C and bacterial isolate L could be described as genus of Bacilli; but, the annotation to level species cannot be determined.

To inform identification results based on barcoding DNA with 16S rRNA sequence, in this paper, we were re-analysis 16S sequence from (Rodiansyah et al., 2021) with additional reference sequences specific on *Bacillus cereus* Group and *Bacillus subtilis* Group. The maximum likelihood (ML) tree showed that both genus Bacilli separated into two groups with a confidence value of 100. The bacterial isolate C is located in one clade with *Bacillus paramycoides* with a confidence value of 66. However, the bacterial isolate L was situated in the same clade with *Bacillus amyloliquefaciens* with a bootstrap value of 36 (Fig. 1).

The genetic distance analysis from genus *Bacillus* shows a significant difference in sequences between-group similarity, about 94%, but within-group similarity shows they are homogeny (Similarity 99%) (Table 7).

Table 7
The 16S rRNA genetic distance within group from bacterial isolate C and bacterial isolate L.

Groups	p-distance	Similarity	SE
<i>Bacillus cereus</i> Group (Isolate C)	0.0033	99.67%	0.0009
<i>Bacillus subtilis</i> Group (Isolate L)	0.0061	99.39%	0.0011
Out of the Group	0.0036	99.64%	0.0016

Chlorella vulgaris cells density and data analysis

The graph in Fig. 2 below shows the influence of the treatments on the microalgae growth from day 0 to 50. The growth data was measured with the calculation of cell density using haemocytometer. In general, microbial consortium treatments have significantly different growth of microalgae compared to control.

In treatment I (control treatment), the pattern of the growth of *C. vulgaris* start from the lag phase (day 0 to 6), then the log phase (day 6 to 12), and finally, the death phase (day 12 to 50) (purple line in Fig. 2). The graph of the microalgae growth in this treatment has a moderate increase from day 2 to 12 that has the highest average cell density reaching 3.5×10^5 cells/mL. After that, the growth of microalgae decreased gradually on day 14 and then levelled off until day 50 that has an average cell density of 3×10^3 cells/mL.

In treatment II, the growth of *C. vulgaris* starts from the lag phase (day 0 to 10), log phase (day 10 to 38), and death phase (day 40 to 50) (green line in Fig. 2). The growth of microalgae in the lag phase decreased slightly from day 0 to 10, in the log phase rose gradually and then climbed sharply, with an average cell density of 2.4×10^6 cells/mL. After reaching the death phase, the growth of microalgae dropped moderately, with an average cell density of 1.9×10^6 cells/mL. In treatment III, the development of *C. vulgaris* start from the lag phase (day 0 to 8); after that, the growth of microalgae fell steadily until day ten, then boom dramatically at the log phase (day 12 to 50) with cells density reaching at 2.7×10^6 cells/mL (red line in Fig. 2). In treatment IV, the growth of *C. vulgaris* starts from the lag phase (day 0 to 10), log phase (day 12 to 38), and death phase (day 40 to 50) (blue line in Fig. 2). At the lag phase, the growth of microalgae decreased slowly then climb dramatically at the log phase with a cell density of 2.1×10^6 cells/mL; finally, there was a fall gradual in the death phase with an average cell density of 1.4×10^6 cells/mL.

The hypothesis calculated with ANOVA t-test shows that the F value > F Table (Table 8); as a result, the hypothesis is accepted, indicated that there is an influence of a consortium of amylolytic bacteria and IAA producing bacteria on the *C. vulgaris* growth.

Table 8
Hypothesis test results with one-way ANOVA t-test

	<i>Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
Between Groups	12.468	3	4.156	16.88	.000
Within Groups	23.136	94	.246		
Total	35.604	97			

The treatments other than control gives a significant influence on the development of the density of *C. vulgaris* cells. The further test with the Least Significant Difference Test (LSD) significant 5% can be seen in Table 8. Treatment III showed the best treatment for increasing *C. vulgaris*, which microbial consortium treatments are significantly different compared to the control treatment (Table 9).

Table 9
Results of LSD 5% from 4 treatments

	Treatment	N	Subset for alpha = 0.05	
			1	2
LSD ^a	C	20	.904	
	C + A	26		1.7417
	C + I + A	26		1.7993
	C + I	26		1.8178
	Sig.		1.00	.619

Discussion

Heterotrophic bacteria are available in various ecosystems, including lake waters (K. Liu et al., 2016). The presence of heterotrophic bacteria in lake waters is influenced by organic material in lake waters that can support bacterial growth (Yuningsih et al., 2014). Heterotrophic bacteria can decompose complex organic compounds containing elements C, H, and N into simpler compounds because they can produce extracellular enzymes (K. Liu et al., 2016). Some bacterial isolates that were successfully isolated in this study prove that the waters from four lakes can supply the nutrition needed by bacteria for supporting their life.

Amylolytic bacteria can gradually degrade amylum in lake waters because amylum can not directly convert amylum into glucose (Rahmansyah & Sudiana, 2010). Initially, amylum will be hydrolyzed into the dextrin compound in the form of polysaccharides. The dextrin is re-hydrolyzed again into oligosaccharides in the form of maltose. Maltose is subsequently converted into monosaccharides in the form of glucose (Brewster, 1953). Our highest amylolytic bacteria from Ranu Pani, based on phenotypic and genotypic characteristics, was identified as *Bacillus amyloliquefaciens*. This species is an amylolytic bacteria that play an essential role as a remodel of organic materials into simpler inorganic components in nature (Waluyo, 2009). Amylolytic bacteria can produce extracellular enzymes to degrading amylum in water (Brown et al., 2008). Amylum that has been degraded into simpler compounds can be utilized by *C. vulgaris* microalgae as a raw material for the photosynthesis process (Brown et al., 2008). The carbon element from carbohydrate and protein decomposition by bacteria in waters can increase the biomass of microalgae *C. vulgaris* (Brown et al., 2008). The essence of carbon can be assimilated in waters in the form of CO₂, which plays a role in the photosynthetic process of microalgae *C. vulgaris* (Hopkin, 2020).

On the other hand, this study also uses the highest potential bacteria producing IAA from Ranu Pani, identified as *Bacillus paramycoides*. The analysis results of variants obtained that IAA-producing bacteria significantly influence the growth of *C. vulgaris*. Further tests explained that the treatment of a consortium of IAA-producing bacteria had the most significant influence on the cell density of *C. vulgaris*.

as the indicator from rate growth. Bacteria produce IAA hormones through gene regulation and tryptophan biosynthesis in microalgae as a precursor for bacteria regulation. Amino acids, such as tryptophan, are easily detected by pairs of organisms in the culture to act as nutritional signals for synthesis IAA with indole 3-glycerol phosphate as the primary substrate (Hopkin, 2020; Vessey, 2016). They live together in waters as mutualism symbiotic. Auxin produced from these bacteria could encourage microalgae cell division processes to increase microalgae biomass (Cooper & Smith, 2015). *Bacillus paramycoides* play a vital role in the decomposition of organic compounds that have a function in the growth of microalgae (Vessey, 2016). This species can hydrolyze amylum, produce H₂S, urease, tryptophan deaminase, indole production, and acid production from mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, and arabinose (Y. Liu et al., 2017). These multiple potencies from *Bacillus paramycoides* showed probably more efficient for stimulating the *C. vulgaris* growth because that bacterium continues to produce IAA in a long period at culture media (Pratt, 1938).

In the microbial consortium, bacteria can reduce the oxygen pressure in the culture, and they can convert complex elements (N, S, P, and C) to become elements ready consumed for algae photosynthesis (Ramanan et al., 2016)(Mujtaba & Lee, 2016). Those elements reduced by bacteria can be used as source components for microalgae photosynthesis; after that, the oxygen as photosynthesis products can be used for bacteria to grow and produce some valuable enzymes and metabolites like auxin as a growth factor. Microalgae can consume this auxin to increase the productivity and quality of biomass *C. vulgaris* (Fuentes, Garbayo, Cuaresma, Montero, Valle, et al., 2016). This complex interaction between bacteria and microalgae has tremendous benefits for cultivating the most appropriate, efficient, and environmentally friendly microbe (Dao et al., 2018). Besides on the biological factor, optimalization of microalgae growth on bioreactor also depends on abiotic factors, like flashing light exposure (Lirofiatillah et al., 2020; Yustinadiar et al., 2020), temperatures (Chakraborty et al., 2016), and optimal nutrients used in the medium (Chandra et al., 2019). In the future, this microbial consortium combined with optimal bioreactor could be considered for the new method for culturing microalgae on the industrial scale that has the potency for biodiesel at a low cost and environment friendly (Allen et al., 2018).

Conclusions

The most potential IAA-producing bacteria identified as *Bacillus paramycoides* from Ranu Pani with an IAA concentration of 158.11 ppm, while the highest amylolytic bacteria has a hydrolyze index of 5.9 identified as *Bacillus amyloliquefaciens*. The microbial consortium shows the effectiveness of stimulating *C. vulgaris* growth compared to control. The microbial consortium with IAA-producing bacteria supplementation was the most effective to enhance microalgae growth, reaching up to 2.7×10^6 cells/mL of cell density on day 50. Future research with broad-scale culture in bioreactor and exploring the specific interaction in co-culture are needed for obtaining high biomass.

Declarations

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Availability of data and materials

The complete data was available in supplementary files

Ethics approval and consent to participate

Not applicable

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable

Authors' contributions

IN carried out the co-culture *C. vulgaris* with microbial consortium, tabulated all data and wrote the manuscript, SP designed this study, SP and AW supervised and reviewed the manuscript, YKB carried out the potential test for IAA-producing bacteria and amylolytic bacteria, AR analyzed the bacterial identification, wrote, and reviewed the manuscript, SP, YKB, and DA collected samples for this study.

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Figures

Figure 1

Phylogenetic tree of C isolate, and L isolate with ML method. *Salmonella enterica* subs. *enterica* Strain LT2 and Ty2 set as out of the group.

Figure 2

C. vulgaris cell growth curve in each treatment. The treatment I (purple line), Treatment II (green line), Treatment III (red line), and Treatment IV (blue line).

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