Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13308- 13318
Received 05 March 2021; Accepted 01 April 2021

Characterization of *Sinirhodobacter sp.,* and *Bacillus zanthoxyli*from Bio methanation plant with emphasis on its plant growth promotion(PGP).

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Key words: Bio methanation plant, Algal biomass, *Sinirhodobacter*, *B.zanthoxyli*, PGP.

Abstract:

Plant growth promoting bacteria have gained significant role in its application as an agricultural input. Since most of the isolates that were investigated for their plant growth promoting potential were sourced from various habitats (terrestrial and marine), the present study was designed to identify and utilize isolates frombio methanation plants, comprising of spent waste from production units (mainly marine algae).The isolates from the samples of Biomethanation plants were identified and screened for their majorpotential PGP activities to assess its suitable applications.. *Sinirhodobactersp.,* and *Bacillus zanthoxyli* was observed to be non-hemolytic bacteria with positive results for N fixation and hydrolytic enzyme production. *B.zanthoxyli* exhibited the trait of solubilizing zinc and the zone of zinc solubilization index; zinc solubilization efficiency for the isolate was 3.5 &350% in (24 hours), 7.75 & 750% in(48 hours). *Sinirhodobacter sp.,* and *B.zanthoxyli* produced 90µg/ml and 176µg/ml of Indole acetic acid (IAA). *B.zanthoxyli* showed the presence of orange halo zone formation in CAS agar medium that is representing positive siderophore production. Italsopossessed ACC deaminase activity by releasing 147.8µmoles/ml of α-ketobutyrate. The optimum pH of both the isolates wasobserved to be 7.00 and optimum temperature for growth was observed to be 37°C for *Sinirhodobacter sp.,* and at 30°C for *B.zanthoxyli.* This identification of two new less studied bacteria could pave way in developing novel value added plant growth promoting products.

Introduction:

Tremendous demand in Biofuels has led to an enlarged interest in renewable energy sources. Biofuels production utilizing biomass conversion has predominantly gained attention, as it is an environment friendly, clean renewable source (Ward *et al.,* 2014). Biogas is produced by the anaerobic digestion or fermentation of biodegradable materials such as agricultural, community based and industrial feedstock's. The application value of biogas and the improvement of biogas production have been well developed since last two decades. The third generation of biofuels based on macro and micro algae is gaining momentum. Macro algae located on the coastline contribute a wide part of the ocean biomass upto approximately 30,000

species. Macro algae are rich in nutritional composition and mostly used as food and feed by the coastal people. Due to their unique properties and demand the development of macro algae cultivation methods was increased drastically. Macro algae are utilized by microorganisms, for their carbon rich constituents present within the cell walls (e.g. Agar, Carrageenan, laminarin, pectin, cellulose, fucoidan,) which help microbes in colonization. The microorganisms present in the cell walls, such as macro algal polysaccharide degrading bacteria help in recycling algal biomass. The biogas production from microalgae is well documented(Ward *et al.* 2014, Fuentes *et al.* 2016, Jard *et al.,* 2013), but the production from macroalgae is less evident, and more research into production is required(Bharathiraja *et al.,* 2018)The use of marine macroalgae biomass reduces difficulties like hypertrophication, exhaustion of resources, and diminution in biodiversity (Theuerl *et al.,* 2019).

The microbiomes of anaerobic digesters are extremely complex with diversified microbial communities that aid in the conversion of biomass into biogas by four sequential processes namely hydrolysis, acidogenesis, acetogenesis/dehydrogenation, and methanogenesis (Theuerl et al., 2019).The sequential run of these steps is progressed by using metabolites or end products of one process as precursors for the next process. The recent understandings of the microbiome of biogas characterized by (Theuerl et al., 2019)as 1.microbiomes are specific to the anaerobic digester. 2.Microbes are defined by the substrate and function of the digester. 3. The identity and function of the isolates in a digester is still not well defined. An improved understanding of the aerobic and anaerobic microbiome of anaerobic digesters including their role and ecological interactions aids in the better development of bio methanation plants. All inclusive study on microbial diversity of anaerobic digesters show that species belonging to the Proteobacteria, Bacteroidetes, Firmicutes, Actionbacteria, Synergistetes and Euryarchaeota are predominant (Houari et al. 2020)Identification of microbial diversity both culturable and non culturable methodologies. through the use of sequencing tools like 16s rRNA sequencing, gene amplification, metagenome, metatranscriptome, and metaproteome gives better understanding on the unculturable microbial diversity and enrichment techniques for isolation of culturable microbes (Lim*et al*., 2020). In our previous study (Ponni *et al*., 2019) taxonomic profiling of biogas producing communities by methyl coenzyme reductase α-subunit (mcrA) gene Amplicon sequencing was highlighted. The understanding of the microbiome helps in determining the conditions for successful identification of suitable or specific microbiome and their application as microbial indicators for upgrading bio methanation, the use of specific single organism/microbial consort in agricultural areas, like to enhance soil quality, crop production, and nutrient/disease management in plants. The present study elucidates identification of microbiome from anaerobic digesters by culturing and 16s rRNA identification and an attempt to studythe plant growth promoting bacterial community for development of a value added biofertilizers in nutrient management for agricultural application.

Materials and methods:

Spent algal biomass collection:

The spent wastes of (*Sargassum*and *Kappaphycus sp.),* macro algae were collected from the production warehouse of T. Stanes and Company limited and recycled for biogas production with the developed Cow dung-seaweed (CDS) inoculum.

Exploration of culturable plant growth promoting microorganisms:

The CDS inoculum and samples from pilot and large scale bio methanation (BM) plant were checked for their microbial activity in aerobic and anaerobic conditions. The isolation of anaerobic bacteria was carried out in sodium thioglycollate (ST) medium and aerobic bacteria in plate count agar (PCA). The CDS inoculum and BM plant samples were serially diluted and pour plated on to the ST medium, incubated in anaerobic jar with gas pak, to maintain complete anaerobic conditions with an incubation period of 7 days. Aerobic organisms pour plated on PCA and incubated at 30°C for 5 days. After incubation, different colonies were marked as CDS MET O1 to 06, CDS M1 to M5 and sequential subcultures were done to obtain pure cultures**.**

Screening for plant growth promoting traits:

Nutrient solubilization:

Phosphate solubilization was tested in Pikovskaya medium (Pikovskaya, 1948); nitrogen fixation in nitrogen free malic acid medium by modified IS 14806:2000 method, Potassium solubilization checked in Alkensandrov agar (Alkansandrov, 1967), zinc solubilization in Zinc oxide agar medium.The degree of solubilization was determined by measuring the zone of solubilization in mm. Zinc solubilization index (ZSI) was calculated by dividing halo zone by colony. The zinc solubilization efficiency was calculated by ZSI multiply with 100. (Vazques *et al* 2000). Silica solubilization was evaluated using silica medium incorporated with magnesium tri silicate with glucose as sole source of carbon. Clear zone around the colonies indicates the Si solubilizing ability of the bacteria.(Naureen et al. 2015)

Plant growth promoting activities:

IAA production was evaluated in 0.1% tryptophan supplemented in peptone water (Loper and Schroth, 1986), ACC deaminase activity was analyzed as per the method of (Penrose & Glick, 2003), Siderophore production was screened in Chrome Azurol S agar medium as defined by (Alexandar *et al.,* 1991). Hydrogen cyanide production tested in Tryptic soy agar medium incorporated with 1% glycine as described by (Correa *et al.,* 2005).

Molecular characterization and biochemical characterization:

The genomic DNA was isolated as specified by (Atashpaz et al. 2010)and the 16S rRNA was

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13308- 13318
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amplified with 27F and 1492R Primer, the amplified product was purified and sequenced. General characteristics of the isolates were checked by examining Gram staining, oxidase, catalase, and fluorescens @ 354nm. Pathogenicity of all the isolates checked in sheep blood agar medium for zone of hemolysis. Based on the hemolysis test, non-hemolytic isolates CDS Met 05 & 06 and CDS M3-2&4 were selected for further investigations

Optimization of growth conditions - pH and temperature for *Sinirhodobacter sp.*, and *Bacillus zanthoxyli*:

The growth conditions of *Sinirhodobacter*, was checked in the standardized medium (Glucose – 10g l^{-1}, $MgSo_4$ -1.25g l^{-1}, KH_2Po_4 – 2g l^{-1}, Yeast extract -2.5g l^{-1}, Peptone - 2.5g l^{-1}) Likewise the *Bacillus zanthoxyli* was studied in (Glucose-8g l^{-1}, Soya peptone -1g l^{-1}, Tryptone-1g l^{-1}, Peptone -1.5 g l^{-1}, KCl- 0.5g l^{-1} $MgSo_4$ - 0.25g l^{-1}, KH_2Po_4 – 0.8 g l^{-1}, NaCl - 2g l^{-1} for pH values (3,5,7 & 9) and temperatures (25°C,30°C,37°C&40°C). Overnight grown cultures of the isolates were inoculated in their selective medium with different pH) and incubated at the respective temperaturesAfter incubation, the optical density was read at 540nm in a spectrophotometer and the optimum growth conditions were determined.

Results:

Eleven isolates were isolated from CDS inoculum and biomethanation plant samples

All the isolates were checked for their plant growth promoting activities (In vitro) and significant results were observed. Maximum solubilization ofphosphate and potassium was observed with CDS M-5 (around 14-20 mm zone) within 24 hours. (CDS M3-2, 4, 5 and 6)marked zinc solubilization with a clear halo zone ranging between 4-22mm.CDS met 03 & CDS M3-5 showed silica solubilization, relatively higher compared to other isolates. N fixation ability was seen in all isolates. CDS M6 & CDS met 03 showed orange halo zone in CAS agar medium indicating positive results for Siderophore production. IAA production was observed in all isolates. No hydrogen cyanide was produced by the isolates. ACC deaminase activity was observed with few isolates (CDS MET 5, 6 CDS M3-2 & CDS M3-6).

Table 01- Preliminary screening of biomethanation plant isolates for PGP traits:

PGPR activity	CDS Met 01	CDS Met 02	CDS Met 03	CDS Met 04	CDS Met 05	CDS Met 06	CDS M3-2	CDS M3-3	CDS M3-4	CDS M3-5	CDS M3-6
Nutrient solubilization											
Phosphate	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	+++	- - -
Zinc	- - -	- - -	- - -	- - -	- - -	- - -	+++		+++	+++	+++
Silica	- - -	- - -	+++	- - -	- - -	- - -	- - -	- - -	- - -	+++	
Potassium	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	+++	- - -
Nitrogen fixation	++	++	++	++	+++	+++	+++	++	++	+++	+++

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PGP activities											
IAA	+	++	++	++	+++	+++	+++	++	++	+++	+++
HCN	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
Siderophore	- - -	- - -	++	- - -	- - -	- - -	+++	- - -	- - -	- - -	+++
ACC deaminase	- - -	- - -	- - -	- - -	+++	+++	+++	- - -	- - -	- - -	+++
Hydrolytic enzymes											
Amylase	- - -	- - -	- - -	- - -	- - -	- - -	+++	- - -	- - -	- - -	+++
Cellulase	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	
Protease	- - -	- - -	- - -	- - -	++	++	+++	- - -	- - -	- - -	+++
Chitinase	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	

{+ : Slightly positive, ++ : Moderate, +++ : Highly positive, - - - : Negative}

Microbial identification:

The microbial analysis of the spent waste of the Bio methanation plant samples resulted in the isolation of 11 isolates. The habitat diversity showed that the isolates were facultative anaerobes and the optimum growth temperatures of the isolates were 37 °C withstanding both aerobic and anaerobic conditions. Morphological studies showed that 7 isolates were gram negative rod shaped organism and 4 gram positive rod shaped organism. The molecular sequencing results and gen bank accession number of the isolates are tabulated in (Table 01). Biochemical characterizations of the isolates aredepicted in (Table 02)

Table 02 – Molecular identification & gen bank accession number.

S.No	Culture code	Source	Culture identified by 16s rRNA sequencing as	Gen bank accession number
1	CDS Met 01	CDS inoculum (Lab scale biomethanation plant)	*Stenotrophomonas acidaminiphila*	MT225950.1
2	CDS Met 02		*E.coli*	MT225951.1
3	CDS Met 03		*Enterobacter sp.,*	NA
4	CDS Met 04		*Exiguobacterium mexicanum*	MT225949.1
5	CDS Met 05		*Sinirhodobacter sp.,*	MT225947.1
6	CDS Met 06		*Sinirhodobacter sp.,*	MT225948.1
7	CDS	Biomethanation	*Bacillus zanthoxyli*	MT225952.1

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	M3-2	plant sample		
8	CDS M3-3		*Bacillus paramycoides*	MT225953.1
9	CDS M3-4		*Bacillus paranthracis*	MT225954.1
10	CDS M3-5		*Klebsiella variicola*	MT225955.1
11	CDS M3-6		*Bacillus zanthoxyli*	MT225956.1

Table 03 – Biochemical characterization of the Biomethanation plant isolates

Biochemical tests	**CDS Met 01**	**CDS Met 02**	**CDS Met 03**	**CDS Met 04**	**CDS Met 05**	**CDS Met 06**	**CDS M3-2**	**CDS M3-3**	**CDS M3-4**	**CDS M3-5**	**CDS M3-6**
Gram staining	G -ve long rod	G -ve short rod	G –ve rod	G +ve rod	G -ve rod	G -ve short rod	G +ve rod	G +ve rod	G +ve rod	G –ve rod	G +ve rod
Oxidase	---	---	---	---	---	---	---	+++	---	---	---
Catalase	+++	+++	---	+++	---	---	+++	+++	+++	---	+++
Fluorescens @ 353nm	---	---	---	---	---	---	---	---	---	---	---
Growth at 37°C	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Lactose fermentation	+++	+++	+++	---	---	---	---	---	----	+++	---
Hemolysis	+++	+++	+++	+++	---	---	---	+++	+++	+++	---

{+++: Positive, ---: Negative}

Characterization of *Sinirhodobacter* and *Bacillus zanthoxyli*:

Nitrogen fixation: The development of blue color in nitrogen free malic acid medium indicated positive results for nitrogen fixation (Figure 01). The quantitative estimation was determined by measuring the optical density of the isolates at 595 nm which showed significant fixation of N fixation (0.212 & 0.186) for *Sinirhodobacter sp.*, compared to the well-established *Azospirillum sp., (0.161).*

Figure 01 –Photograph showing Nitrogen fixation ability of *Sinirhodobacter sp.*,

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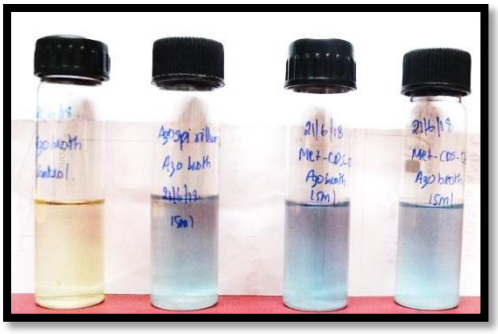

B.zanthoxyli showed a clear zone of solubilization 10mm in 24 hours, 27mm in 48 hours on plate assay with zinc oxide agar. (Figure 02).The zone of zinc solubilization index and zinc solubilization efficiency for the isolate was 3.5, 350% in 24 hours, 7.75, 750% at 48 hours.

Figure 02 –Photograph showing Zinc solubilization zone by *B.zanthoxyli* on Zinc oxide agar.

Indole acetic acid (IAA), Siderophore and ACC Deaminase activity:

The isolates (Sinirhodobacter *sp.,* and *Bacillus zanthoxyli)* produced 90µg/ml and 176µg/ml of IAA respectively. *Bacillus zanthoxyli* showed orange halo zone formation in CAS agar medium indicating siderophore production.Bacillus zanthoxyli possessed ACC deaminase activity by releasing 147.8µmoles/ml of α-ketobutyrate.

Optimized growth conditions:

The optimum pH of *Sinirhodobacter sp.,* and *Bacillus zanthoxyli* was 7.00 (Figure-04) and both the isolates were able to thrive at alkaline pH 9.00 and temperature of40°C . The optimum temperature for maximum growth was observed to be 37°C for *Sinirhodobacter sp.,* and at 30°Cfor *B.zanthoxyli*(Figure-05)

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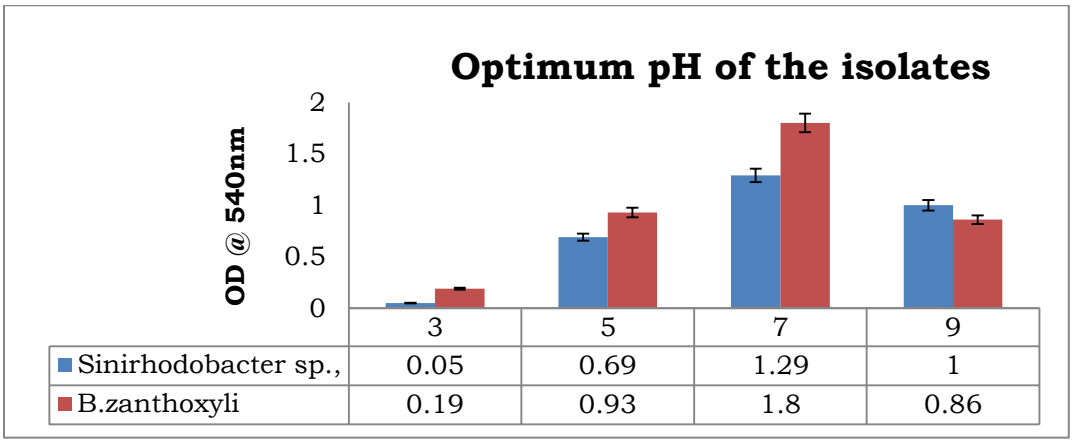
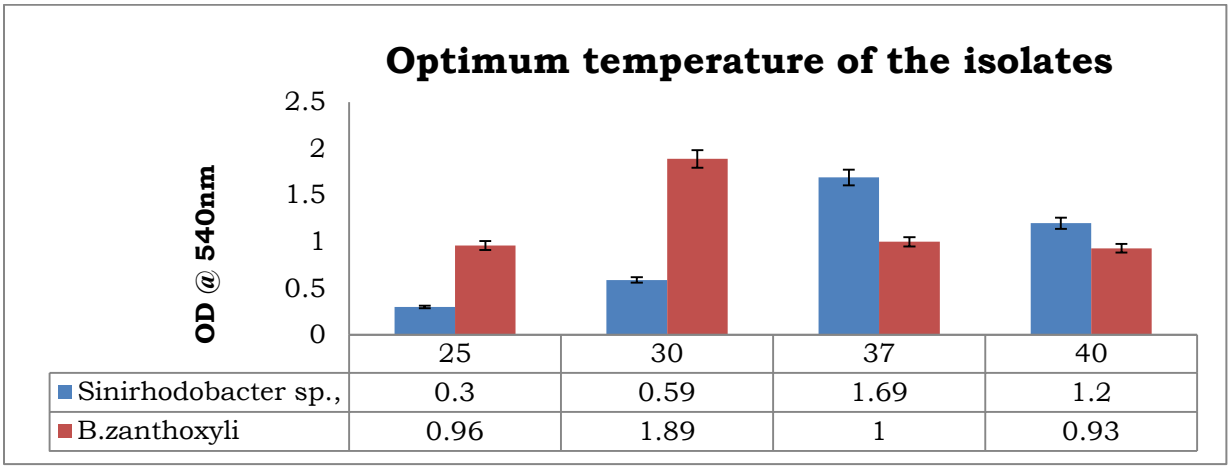
Figure 04- Optimized for best pH



	3	5	7	9
Sinirhodobacter sp.,	0.05	0.69	1.29	1
B.zanthoxyli	0.19	0.93	1.8	0.86

Figure 05- Optimized for best Temperature:



	25	30	37	40
Sinirhodobacter sp.,	0.3	0.59	1.69	1.2
B.zanthoxyli	0.96	1.89	1	0.93

Discussions:

With a huge demand, the availability of plant growth promoting biofertilizers from one source like plant rhizosphere will not be sufficient for agriculture applications, exploitation of other sources are to be identified and studied. The other source that can be utilized is the diversified marine resources with the presence of 9000 species of macro algae. Researchers found the secretion of various fine chemicals, micro nutrients and plant growth promoting hormones, cytokinin, gibberellins, trace elements, vitamins, amino acids and antibiotics(Tay *et al*., 1987,Thirumaran*et al*., 2009, Wajahatullah *et al*., 2009). Based on the significant properties and applications of macro algae, the present study was carried out using the spent macro algal wastes from production unit for bio methane conversion and isolation of PGP microorganisms from the biomethanation plants and characterization of their activities. The isolates (11) were obtained from the bio methanation plant samples include 4 isolates (*Stenotrophomonas, Enterobacter, E.coli* and *Klebsiella*) thatbelongs to class gammaproteobacteria, 2 *Sinirhodobacter sp.*, belonging to alphaproteobacteria of Proteobacteria phylum whereas other 5 isolates belonging to class Bacilli of Firmicutes phylum. Most investigated bio methanation plant microbiome consists of *Firmicutes* and *Actinobacteria* phylum for fermentation process

& the acdiogenicand acetogenic process involves *Bacteroidetes, Actinobacteria, chloroflexi, Firmicutes, Proteobacteria, Syngergistetes, Coacimonetes*whereas*Archaea* involves in methanogenic process(Theuerl *et al.,* 2019) . Most organisms associated with macro algae also includes the above phyla (Venter *et al*., 2004; Rusch*et al.,* 2007, Giovannoni & Stingl, 2005; Burke *et al.,* 2011a). In this study all the isolates were screened for plant growth promoting attributes as,Plant growth promotion by bacteria takes place by direct and indirect mechanism. They help plant by fixing atmospheric nitrogen, solubilizing insoluble nutrients like phosphate, zinc, potassium, by production of organic acids, by producing growth hormone like indole acetic acid and gibberellins directly and produce ACC deaminase production to regulate ethylene, induced systemic resistance ISR, antibiosis and production of metabolites by indirect mode(Miransari 2014).Mostly all isolates showed significant PGP activities. Based on the hemolytic activity, two isolates *Sinirhodobacter sp.*, and *Bacillus zanthoxyli* were investigated further. *Sinirhodobacter sp.,* observed to be a new isolate and able to fix nitrogen &produce IAA. The isolate is closely related to phototrophic*Rhodobacter sp.,* and no plant growth promoting attributes research studies were available. The research on one isolate *Sinirhodobacter hankyongi sp. nov*., a novel denitrifying bacterium from sludge samples was isolated by (Young *et al* 2020) was studied. Likewise Bacillus zanthoxyli showed positive for zinc solubilization with a clear halo zone (28mm zone), ACC deaminase 147.8µmoles/ml of α-ketobutyrate& IAA production (176µg/ml of IAA). Previous study state that most PGP bacteria produce ACC deaminase and IAA which degrades ACC as an ethylene precursor, and promotes plant growth. A study conducted by (Usmonov *et al* 2021) state that *Bacillus zanthoxyli* was able to suppress the soil borne pathogens and rendered less sensitivity against saline stress. Since the two isolates are explored less for plant growth promoting attributes, their effect on combating plant pathogens in green house experiments and for feasible development of bioformuation for commercial purpose will be investigated in future..

References:

1. Bharathiraja, B., Sudharsana, T., Jayamuthunagai, J., Praveenkumar, R., Chozhavendhan, S., & Iyyappan, J. (2018). Biogas production – A review on composition, fuel properties, feed stock and principles of anaerobic digestion. *Renewable and Sustainable Energy Reviews*, *90*(July), 570–582. https://doi.org/10.1016/j.rser.2018.03.093
2. Fuentes, J. L., Garbayo, I., Cuaresma, M., Montero, Z., González-Del-Valle, M., & Vílchez, C. (2016). Impact of microalgae-bacteria interactions on the production of algal biomass and associated compounds. *Marine Drugs*, *14*(5). https://doi.org/10.3390/md14050100
3. Penrose, D. M., & Glick, B. R. (2003). Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiologia Plantarum*, *118*(1), 10–15. https://doi.org/10.1034/j.1399-3054.2003.00086.x
4. Theuerl, S., Klang, J., & Prochnow, A. (2019). Process disturbances in agricultural biogas production—causes, mechanisms and effects on the biogas microbiome: A

review. *Energies*, *12*(3). https://doi.org/10.3390/en12030365

5. Ward, A. J., Lewis, D. M., & Green, F. B. (2014). Anaerobic digestion of algae biomass: A review. *Algal Research*, *5*(1), 204–214. https://doi.org/10.1016/j.algal.2014.02.001
6. Jard, G., Dumas, C., Delgenes, J.P., Marfaing, H., Sialve, B., Steyer, J.P., Carrére, H (2013). Effect of thermochemical pretreatment on the solubilization and anaerobic biodegradability of the red macro alga Palmaria palmate. Biochemical Engineering Journal, 79, pp.253-258.
7. Aleksandrov, V. G., Blagodyr, R. N., &Ilev, I. P. (1967). Liberation of phosphoric acid from apatite by silicate bacteria. *Mikrobiol Z*, *29*(11), 1-1.
8. Loper, J. E., & Schroth, M. N. (1986). Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. *Phytopathology*, *76*(4), 386-389.
9. Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya*, *17*, 362-370.
10. Tay, S. A. B., Palni, L. M. S., & MacLeod, J. K. (1987). Identification of cytokinin glucosides in a seaweed extract. *Journal of Plant Growth Regulation*, *5*(3), 133-138.
11. Arumugam, R., &Anantharaman, P. (2009). Effect of seaweed liquid fertilizer on growth and pigment concentration of Abelmoschus esculentus (l) medikus. *Journal of Agronomy*, *2*(2), 57-66.
12. Usmonov, A., Yoo, S. J., Kim, S. T., Yang, J. S., Sang, M. K., & Jung, H. W. (2021). The Bacillus zanthoxyli HS1 Strain Renders Vegetable Plants Resistant and Tolerant against Pathogen Infection and High Salinity Stress. *The Plant Pathology Journal*, *37*(1), 72.
13. Indian Standard, 14807:2000. Phosphate solubilizing bacterial inoculants (PSBI)-Specification. Bureau of Indian Standards, New Delhi, India, 1–4 pp.
14. Alexander, D. B., &Zuberer, D. A. (1991). Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biology and Fertility of soils*, *12*(1), 39-45.
15. Lee, Y. W., Hoang, T. S., Rhee, M. S., Lee, S. Y., &Im, W. T. (2020). Sinirhodobacterhankyongi sp. nov., a novel denitrifying bacterium isolated from sludge. *International journal of systematic and evolutionary microbiology*, *70*(1), 668-674.
16. Giovannoni, S. J., & Stingl, U. (2005). Molecular diversity and ecology of microbial plankton. *Nature*, *437*(7057), 343-348
17. Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. A., ... & Smith, H. O. (2004). Environmental genome shotgun sequencing of the Sargasso Sea. *science*, *304*(5667), 66-74
18. Rusch, D. B., Halpern, A. L., Sutton, G., Heidelberg, K. B., Williamson, S., Yooseph, S., & Venter, J. C. (2007). The Sorcerer II global ocean sampling expedition: northwest Atlantic through eastern tropical Pacific. *PLoS Biol*, *5*(3), e77.
19. Menaa, Farid, P. A. U. I. Wijesinghe, Gobika Thiripuranathar, Bushra Uzair, Haroon Iqbal, Barkat Ali Khan, and Bouzid Menaa. 2020. "Ecological and Industrial

Implications of Dynamic Seaweed-Associated Microbiota Interactions." *Marine Drugs* 18(12):1–25. doi: 10.3390/md18120641.

20. Burke, Catherine, Torsten Thomas, Matt Lewis, Peter Steinberg, and Staffan Kjelleberg. 2011. "Composition, Uniqueness and Variability of the Epiphytic Bacterial Community of the Green Alga Ulva Australis." *ISME Journal* 5(4):590–600. doi: 10.1038/ismej.2010.164.
21. Ponni Keerthana, K., S. Radhesh Krishnan, S. Ragunath Sengali, R. Srinivasan, N. Prabhakaran, G. Balaji, M. Gracy, and K. Latha. 2019. "Microbiome Digital Signature of MCR Genes – an in Silico Approach to Study the Diversity of Methanogenic Population in Laboratory-Developed and Pilot-Scale Anaerobic Digesters." *Access Microbiology* 1(5). doi: 10.1099/acmi.0.000044.
22. Khan, W., Rayirath, U.P., Subramanian, S. *et al.* Seaweed Extracts as Biostimulants of Plant Growth and Development. *J Plant Growth Regul* 28, 386–399 (2009). https://doi.org/10.1007/s00344-009-9103-x
23. Atashpaz, Sina, Sajjad Khani, Abolfazl Barzegari, Jaleh Barar, Sepideh Zununi Vahed, Reza Azarbaijani, and Yadollah Omidi. 2010. "A Robust Universal Method for Extraction of Genomic DNA from Bacterial Species." *Microbiology* 79(4):538–42. doi: 10.1134/S0026261710040168.
24. Donate-Correa, Javier, Milagros León-Barrios, and Ricardo Pérez-Galdona. 2005. "Screening for Plant Growth-Promoting Rhizobacteria in Chamaecytisus Proliferus (Tagasaste), a Forage Tree-Shrub Legume Endemic to the Canary Islands." *Plant and Soil* 266(1–2):261–72. doi: 10.1007/s11104-005-0754-5.
25. Naureen, Zakira, Muhammad Aqeel, Muhammad Nadeem Hassan, Syed Abdullah Gilani, Nahla Bouqellah, Fazal Mabood, Javid Hussain, and Fauzia Y. Hafeez. 2015. "Isolation and Screening of Silicate Bacteria from Various Habitats for Biological Control of Phytopathogenic Fungi." *American Journal of Plant Sciences* 06(18):2850–59. doi: 10.4236/ajps.2015.618282.
26. Vazquez, P., G. Holguin, M. E. Puente, A. Lopez-Cortes, and Y. Bashan. 2000. "Phosphate-Solubilizing Microorganisms Associated with the Rhizosphere of Mangroves in a Semiarid Coastal Lagoon." *Biology and Fertility of Soils* 30(5–6):460–68. doi: 10.1007/s003740050024.
27. Houari, Abdelaziz El, Magali Ranchou-Peyruse, Anthony Ranchou-Peyruse, Rhizlane Bennisse, Radia Bouterfas, Maria Soledad Goni Urriza, Abdel-Ilah Qatibi, and Rémy Guyoneaud. 2020. "Microorganisms Abundance and Diversity in Municipal Anaerobic Sewage Sludge Digesters From." *Processes* 8(1284):1–22.

Funding Information:

The research project was funded by T.Stanes and company limited, Coimbatore.

Acknowledgment:

We acknowledge Dr.Brindha (Microbiologist, T. Stanes and Company Limited) for proof-reading the manuscript.

Conflicts of interest:

The authors declare that there are no conflicts of interest.