Effect of duration of culture period on the agar yield and gel strength of Gracilaria dura C. Agardh (Gracilariaceae, Rhodophyta) at Saurashtra coast, Gujarat, India

Monica Gajanan Kavale (mgkavale@csmcri.res.in)
CSIR- Central Salt & Marine Chemicals Research Institute
Ramavatar Meena
Central Salt and Marine Chemicals Research Institute
V. Veeragurunathan
CSIR- Central Salt & Marine Chemicals Research Institute
M Persis
Academy of Scientific and Innovative Research (AcSIR)

Research Article

Keywords: Gracilaria dura, Culture period, Agar yield, Gel strength, Monoline

Posted Date: May 9th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1613162/v1

Abstract

Gracilaria dura J. Agardh is a promising source of agar and agarose and have potential for commercial farming in India. Due to moderate to strong wave action along the west coast of India, the tube-net and floating monoline methods are recommended for commercial cultivation of *G. dura*. In order to optimized the culture period, for augmented biomass production and better quality of agar, *G. dura* was cultivated by floating monoline method with different culture period such as 15, 25, 35, 45 and 55 days cycle^{-1} during December, 2018 to April, 2020. It was observed that the Daily Growth Rate (DGR), progressively decreased with increase in the duration of culture period. Among the culture period analyzed, the highest DGR (8.47 ± 2.29%) and biomass yield (233.3 ± 82.48 g.fr.wt m^{-1}) was observed at 15 and 35 days culture period respectively. Both biomass yield and DGR significantly ($p < 0.05$) varied between growth period tested. Higher agar yield (34.5 ± 0.71%) and gel strength (1606.5 ± 27.58 g cm^{-2}) was recorded at 35 days and 25 days culture period respectively. However, gel strength did not vary significantly ($p > 0.05$) between different culture periods and agar yield, whereas the ash content was varied significantly ($p > 0.05$) between different culture period. Minimal culture period maintained the vegetative phase of *G. dura* and reducing the maturity (i.e. sporulation phase) which resulted higher biomass yield and DGR. Harvests cycle also reduced while increasing culture period. Therefore, the culture period of 25–35 days was found ideal for commercial farming of *G. dura* along the Saurashtra coast, India.

1. Introduction

The genus *Gracilaria* Greville is the world's third largest maricultured seaweed accounted for 3.6 million tones production during 2019 worth of USD 2 billion. Worldwide, *Gracilaria* spp. are commonly utilized for agar production, animal feed and also consider as human food (FAO 2021). In India, among 33 reported *Gracilaria* species, *G. debilis* (Forsskål) Børgesen, *G. edulis* (S.G. Gmelin) P.C. Silva, *G. salicornia* (C. Agardh) E.Y. Dawson, *G. verrucosa* (Hudson) Papenfuss have been currently utilised by agar industries (Ganesan et al. 2017). In addition to these species, *G. dura* C. Agardh has been emerging as a direct preferred source of high gel strength agarose (1% gel > 1900 g cm^{-2}) (Meena et al. 2014; Veeragurunathan et al. 2015a, b; Mantri et al. 2020).

G. dura is growing in inter-tidal to sub-tidal waters on sandy, muddy and rocky substrata. It is native to Saurashtra, west coast of India (Kavale et al. 2018). The cultivation trials of *G. dura* have been carried out since 2009 along the west coast of India, especially on Gujarat coast. Here the cultivation is possible only during the month of October-April; the stormy weather during monsoon (June-September) restricts the cultivation activity. Further, the bamboo raft method and off-bottom monoline method is not suitable in Gujarat coast because only bay areas are available for cultivation with moderate to strong wave action. Therefore, only tube-net and floating monolines methods are suitable for west coast of India. As *G. dura* is restricted in occurrence, the availability of seed material for commercial cultivation is the major bottle neck. Under these circumstances, the advantage of using monoline method over tube-net method is beneficial as it requires less seed material. For 25-meter-long tube-net required minimum 10kg of fresh weight seed whereas floating monoline needed only 1-2kg fresh weight of seed. Further, floating monoline method showed comparatively higher DGR than tube-net method (Kavale et al. 2021). The only disadvantage was observed in floating monoline was drifting during pre- monsoon conditions (March-April) due to strong wave and wind actions. Therefore, it is recommended that the floating monoline method can be used during onset of cultivation activity i.e. October to February and switched to tube-net during the month March-April (Kavale et al. 2021).

In addition to the methods of cultivation, duration of culture period and agar content are other important aspect of commercial farming. Generally, 40–45 days of culture period was found suitable for *Kappaphycus alvarezii* in south east coast of India (Eswaran et al. 2002) but it may vary according to the species as, 60 days of cultivation period was recommended for various *Gracilaria* spp. cultivated along the south east coast of India (Veeragurunathan et al. 2019). For sustainable commercial farming of *G. dura*, it is essential to optimise the culture period as it directly correlates with the biomass production; as the number of growth cycles increases the production also upsurges. Several studies have been revealed that the agar content could be influenced by the various factors viz. species used for farming, its life stage, habitat, nutrient availability, seasonality, environmental parameters and methods of cultivation etc. (Marinho-Soriano and Bourret 2003; Lee et al. 2016; Rejeki et al. 2018). The present study was carried out with a view to optimise the culture period of *G. dura* through floating monoline method for higher biomass production and to obtain better quality of agar.

2. Materials And Methods

2. 1. Study area:

The initial seed material of *G. dura* was collected from CSMCRI's experimental farm at Simar located in Gir, Somnath District of Gujarat State, India. The experimental trials were carried out at Rajpara village (20.78°N, 71.17°E) approximately 4 km away from Simar (Fig.1). Rajpara is a bay area with sandy bottom and have moderate to strong wave action.

2. 2. Experimental cultivation trials:

The cultivation experimental trials of *G. dura* were done through the floating monolines to assess the effect of duration of culture period (15, 25, 35, 45 and 55 days cycle^{-1}) on the agar characteristics. The floating monoline is the simplest method of cultivation. In this method, 5 to 10g of seed material of *G. dura* was tied with a nylon braider and the seeded braiders were tied with a knot at equidistance to the polypropylene rope of 25-meter length with an initial weight of monoline is 1-1.5kg fresh weight (Fig.2.a). These seeded monolines were then placed in the bay area. To maintain the bouncy, 5 floaters are tied to each seeded polypropylene rope and anchored with approximately 50kg of anchor stones at both the ends. Additionally, 30kg of anchor stones were tied intermediately 5-meter intervals to seeded monolines to avoid displacement. Totally, 30 seeded monolines of each culture period (15-55) were placed horizontally with submerged condition about 10cm below the seawater surface. The monolines were arranged parallel to the wave action with 10-meter distance from each other. The biomass of *G. dura* was harvested on 15th, 25th, 35th 45thand 55th day in respective culture period (Fig.2.b). The experiment of cultivation was carried out in different culture period with five harvest cycles during December, 2018 to April, 2020. Each

The DGR of floating monoline was calculated with the following formula (Dawes 1995).

$$\mathrm{DGR}(\%) = \frac{ln(\mathrm{Wf} - \mathrm{Wi})}{\mathrm{t}} * 100$$

W_f –final fresh weight at day; W_i – initial fresh weight; t - number of culture days

The yield (Y) was calculated by following formula (Mantri et al. 2020)

Y (kg fresh weight m^{-1}) = (Wf–Wi /L).

W_f –final fresh weight; W_i – initial fresh weight; L – length of the floating monoline

2. 3. Extraction of agar from cultivated *G. dura*:

With the aim to examine the effect of duration of culture period of *G. dura* on agar characteristics (yield, gel strength and ash content) the biomass of 30 monolines of each growth period (15-55 days) was harvested in each harvest cycle. The thalli were clean several times with seawater to remove sand, epiphytes and other debris. The whole thalli were taken for shade drying. The agar extraction was carried out in triplicate separately for the biomass of each culture period of *G. dura* following the method described by Meena et al. (2007). In brief, each 10g sample was soaked in water for 1 hour at ambient temperature, and subjected to 10% aqueous NaOH treatment for 2 hours at 70°C. This alkali treated biomass was washed with water to remove excess alkali, and then autoclaved at 120°C for 90 minutes. The hot extract obtained was homogenised using a pulveriser and vacuum filtered over a Celite bed. The gel was freeze-thawed and the agar thus obtained was then dried and ground using mortar and pestle. Agar yield was calculated on the dry weight basis of *G. dura* containing nil moisture. The gel strength measurements were done using 1.5 % w/v agar gels on a Gel Tester (Kiya Seisakusho, Ltd., Tokyo, Japan). The gelling and melting temperatures of agar gels were measured as described by Meena et al. (2007). The ash content was estimated in the residue that was obtained after igniting the agar sample at 550^{o}C for 4 hours. Infrared spectra (IR) was recorded on a Perkin-Elmer Spectrum GX, FT-IR System, by taking 2.0 mg of agar in 600 mg of KBr and compared with control agar (Hi Media).

2. 4. Statistical analysis

One-way ANOVA was performed to analyse the variations in growth and agar properties of *G. dura* tested in various culture period (15-55 days) and Tukey's multiple comparison tests was used whenever a significant range (p□0.05) was observed in ANOVA and the differences were given in different small letters. All the statistical analyses were carried out with InfoStat software (version 2020).

3. Results

3.1. Growth parameters

3.1.1. Daily Growth Rate

The average DGR of different culture period is given in figure 2. The average DGR ranged from 3.21±0.69 to 8.47±2.29 % Day^{-1}. Among the culture period assessed, the culture period of 15 days showed the highest growth rate 8.47±2.29 % Day^{-1} followed by 25, 35, 45 and 55 days culture periods in decreasing order. (Fig.3). It was observed that while increasing duration of culture period, DGR was declined in consecutive culture period and it was varied significantly (F=237.33, p=0.001). Post ANOVA Tukey's test results demonstrated that daily growth rate recorded in 15days culture period was significantly varied with other culture period tested. Similarly, DGR recorded in 25and 35days culture period was significantly varied with 15, 45and 55 days culture period. No variations observed in DGR recorded in 45 and 55 days culture period.

3.1.2. Biomass yield

The average biomass yield of *G. dura* in different culture period is given in figure 4. The average biomass yield ranging from 175.33±94.25 to 233.36±82.48 g.fr.wt m^{-1}. The highest average biomass yield (231.33±82.48 g.fr.wt m^{-1}) was observed in 35 days culture which followed by 25 days culture period (223.6±56.4 g.fr.wt m^{-1}) while the lowest biomass yield was obtained in 15 days culture period (175.33±94.25 g.fr.wt m^{-1}) (Fig.3). It was observed that while increasing duration of culture period, biomass yield was increased up to 35 days and reduced afterwards and it was varied significantly (F=6.14, p=0.001). The Post hoc Tukey's test revealed that biomass yield obtained from 15 days and 55 days culture period was differed significantly with biomass yield obtained from different culture period.

3. 2. Agar Extraction and Characterization:

3. 2. 1. Yield

The agar yields were ranging from 26±1.14% to 34.5±0.71 % (table 1). The maximum yield was obtained from the growth cycle 35 (34.5±0.71%) followed by 25 (33.19±0.26%) and 55(32.0.5±0.78%). The results of this investigation revealed that the yield of agar content decreased with reduction in culture period. Agar yield was significantly (F=16.5, p-value=0.0047) differed with different culture period. The post Tukey's multiple comparison test revealed that agar yield observed in culture period 35 and 15 were differed with culture period 25, 45 and 55.

3. 2. 2. Gel Strength

In this study, the highest gel strength (1606.5±27.58g cm^{-2}) was obtained from 25 days culture period while the lowest (1367.5±163.34g cm^{-2}) was obtained form 15 days culture period (Table 1). There was no significant ($p>0.05$) difference observed in the gel strength of all the growth cycles The gelling and melting temperatures of agar gels of all *G. dura* samples from different culture periods were recorded as 39 ± 1°C and 89 ± 1°C respectively.

Table 1
Characterization of Agar in different culture periods (n = 30) for 5 harvest cycles. Letters indicated post-ANOVA Tukey's test results for differences found indifferent harvest periods ($p < 0.05$). Same letter indicates no significant differences ($p > 0.05$).

Culture periods	Agar yield (%)	Gel strength ($g\ cm^{-2}$)	Ash content (%)
15 days	26 ± 1.14^{c}	1367.5 ± 163.34^{a}	1.11 ± 0.02^{c}
25 days	33.19 ± 0.26^{ab}	1606.5 ± 27.58^{a}	1.26 ± 0.01^{b}
35 days	34.5 ± 0.71^{a}	1508.5 ± 0.71^{a}	1.36 ± 0.05^{ab}
45 days	28.5 ± 2.12^{bc}	1507.5 ± 150.61^{a}	1.44 ± 0.03^{a}
55 days	$32.0.5 \pm 0.78^{ab}$	1457 ± 24.75^{a}	1.41 ± 0.01^{a}

3. 2. 3. Ash content

The ash content of agar samples of *G. dura* varied from 1.11±0.02 to 1.44±0.03 % (table 1). The maximum ash content of agar (1.44±0.03 %) was observed in culture period of 45 days, followed by 55,35,25 and15 days in declining order (Table 1) and it varied significantly (F=16.5, p=0.0047). The post Tukey's multiple comparison test revealed that agar yield observed in 15 days culture period was significantly different from the rest of the culture periods.

3.2.4. FTIR

Fig 5 shows FTIR spectra of agar extracted from *G. dura* and control agar (Hi Media, Mumbai). FTIR spectrum of *G. dura* agar shows characteristic IR peaks at ~930 cm^{-1} (3,6-anhydrogalactose), ~1073 (bending vibration of glycosidic linkage, C-O-C), ~1225 cm^{-1} (sulphate ester), and ~3442 cm^{-1} for hydroxyl group of agar. These spectra were found to be similar to that of control agar used as a reference sample in this study, revealing that the agar produced in this study has an identical chemical structure of Hi Media agar (control).

4. Discussion

Worldwide, about 91% of agar was manufactured from *Gracilaria* spp. and 9% obtained from *Gelidium*, *Gelidiella* and *Pterocladia* (Porse and Rudolph 2017). *Gracilaria* is being farmed mainly through vegetative propagation in pond co-cultured with other animal species mostly with shrimps and abalone with floating raft and off-bottom long line method with a growth rate reported from 1.21 to more than 10% (Cirik et al. 2010; Baloo et al. 2011; Yang et al. 2015; Diatin et al. 2020). In India, the experimental cultivation of *Gracilaria* species namely, *G. debilis*, *G. dura*, *G. edulis* and *G. verrucosa* was attempted by using various methods which includes off-bottom long line method, single rope floating technique, spore culture technique, floating bamboo raft method, polypropylene net method, hanging rope technique, net bag method and net pouch method (Table 2) (Subbaramaiah and Thomas 1990; Jayasankar and Varghese 2002; Padhi et al. 2011; Veeragurunathan et al. 2015a,b, 2016, 2019; Mantri et al. 2020).

Table 2
DGR recorded in cultivated *Gracilaria* species in India

Name of the species	Method of cultivation	Culture period	DGR	Name of the place	Reference
Gracilaria dura	Spore-based seedling through bamboo raft method	45 days	4.67% day^{-1}	Port Okha, Gujarat	Mantri et al. 2009
G. dura	Polypropylene net method	45 days	3.75% day^{-1}	Mandapam, Tamil Nadu	Veeragurunathan et al. 2015a
G. dura	Floating Bamboo Raft method	45 days	2.61% day^{-1}	Mandapam, Tamil Nadu	Veeragurunathan et al. 2015a,b
G. dura	Net bag method	45 days	3.17% day^{-1}	Mandapam, Tamil Nadu	Veeragurunathan et al. 2015a
G. dura	Net pouch	45 days	0.985% day^{-1}	Mandapam, Tamil Nadu	Veeragurunathan et al. 2015a
G. dura	Hanging Rope Technique	45 days	0.726% day^{-1}	Mandapam, Tamil Nadu	Veeragurunathan et al. 2015a
G. dura	Tube-net method	45 days	1.88–3.30% day^{-1}	Simar, Gujarat	Mantri et al. 2020
G. debilis	Floating Bamboo raft	60days	3.59–4.17% day^{-1}	Mandapam, Tamil Nadu	Veeragurunathan et al. 2019
G. edulis	Single Rope Floating Technique (SRFT)	90–120 days	5.48% day^{-1}	Krusadai island, Tamil Nadu	Subbaramaiah and Thomas 1990
G. edulis	Bamboo raft method	60days	5.82% day^{-1}	Thonithurai, Tamilnadu	Veeragurunathan et al.2016
G. edulis	Through spores in nylon bag	22 days	7.73% day^{-1}	Narakkal, Kochi, Kerala	Jayasankar and Varghese 2002
G. verrucosa	Bamboo Raft method	30 days	8.65–8.96% day^{-1}	Chilika Lake, Odisha	Padhi et al. 2011

For *G. dura* the maximum DGR (3.17% day^{-1}) was recorded by Veeragurunathan et al. (2015a) through polypropylene net method from south east coast of India; while Mantri et al. (2009; 2020) reported 4.67% day^{-1} for bamboo raft method and 1.88 ± 0.23% day^{-1} to 3.30 ± 0.25% day^{-1} from tubular net method along the west coast of India (Table 3). The DGRs in the present study (1.74 ± 0.44 to 12.22 ± 2.05% Day^{-1}) were either similar or higher than the DGRs reported for various *Gracilaria* species in India as well as in the foreign countries. Ganesan et al. (2011) noted that the methods of cultivation had spacial and temporal variation affect on the biomass yield and growth rate in red algae. In the present study, the highest biomass yield was found in 35 days culture period followed by 15 and 45 days. The lowest biomass yield was recorded in 55 days of culture period in the month of April. The probable reason for loss of biomass is the drifting, prone to strong wind and wave action of pre-monsoon conditions. There was no specific trend observed among DGR and biomass yield with respect to the growth cycles. Temperature is the crucial factor for growth of various *Gracilaria* spp. The ambient temperature recorded during culture period (December 2019 to April 2020) was ranged from 17.9–34°C (minimum to maximum) with an average range of 22.4–27.6°C. During 5 months of cultivation experimental period the highest biomass yield and DGR were observed in the range of February > January > December where the average ambient temperature was 22.4–23.7°C while the lowest biomass yield and DGR was recorded April > March at 25.9–27.6°C.

Table 3
Comparison of present study data with other Indian agarophytes

Sr. No.	Name of Algae	Area of cultivation	Method of cultivation	Agar Yield (%)	Gel Strength (g cm^{-2})	Gelling Temperature (°C)	Melting temperature (°C)	Ash Content (%)	References
1	*Gelidium pusillum*	Thonithurai Palk Bay, Tamil Nadu	Bamboo Raft	6.9	1800	36	88	≤ 1	Veeragurunathan et al. 2018
			Net bag	9.5	2100	35	88	≤ 1	
2	*Gelidiella acerosa*	Krusadai island Gulf of Mannar, Tamil Nadu	Suspended stone	16	2400	41	86	-	Ganesan et al. 2015
3	*Gracilaria dura*	Simar, Gujarat	Tube net	15.50-19.15	1507.67 -2087.67	-	-	-	Shah et al. 2021
4	*Gracilaria dura*	Rajpara, Gujarat	Monoline	26–34	1367.5-1606.5	39	89	1.11–1.14	Present study

The quality of agar is depending upon the species used for farming, season, habitat, nutrient availability, environmental parameters, methods of cultivation and extraction (Marinho-Soriano and Bourret 2003, Rejeki et al.2018). In the present study, culture period was optimised based on agar characteristics of various culture period from 15–55 days. According to the agar data shown in Table 2, it was confirmed that the culture period of 25 and 35 days showed the highest gel strength and agar yield. It was observed that the agar gel strength was not significantly affected by duration of culture period however, agar yield showed significant difference. The culture period of 15 days showed highest DGR and lowest agar yield. There was no significant difference in agar yield of culture period of 25, 35 and 55 days. Therefore, for the commercial farming the growth cycle of 25 to 35 days should be preferable. Veeragurunathan et al. (2015b) observed increase in period of cultivation resulted in decrease in DGR in *G. dura* cultivated along the south east coast of India. Further, he also notated that 15 days of cultivation period showed highest DGR. Although our results corroborate with Veeragurunathan et al. (2015a,b) observations in *G. dura* cultivation, the agar yield was significantly lower for 15 days of culture period than 25–35 culture period. It was apparent from various research communications that season, habitat, species, geography and environmental parameters, methods of cultivation could influence the agar yield and gelling properties. However, in addition to these factors, duration of growth cycle also proved to be one of the imperative factor.

In India, 32 *Gracilaria* species reported however, there were limited reports available among Indian agarophytes for higher quality agar where hydrocolloid characterisation was made with respect to methods and culture periods (Table 2). Higher quality agar was reported from cultivated material of *Gelidium pusillum* (Veeragurunathan et al. 2018), *Gelidiella acerosa* (Ganesan et al. 2015), *Gracilaria dura* (Shah et al. 2021). The present study result demonstrated that the higher agar yield of 26–34% as compared to previous reported value in *G. dura* (Shah et al. 2021), *Gelidiella acerosa* (Ganesan et al. 2015) and Gelidium pusillum (Veeragurunathan et al. 2018). However, gel strength was comparatively lesser than *G. dura* reported by Shah et al. (2021) which cultivated by tube net method at Simar, Gujarat and *Gelidiella acerosa* by Ganesan et al. (2015), cultivated by suspended stone method and *Gelidium pusillum* by Veeragurunathan et al. (2018), and cultivated by net bag and bamboo raft method. In this present study, agar yield was significantly differed with different culture periods. Variations in agar yield and gel strength was depended on species variation and aging and reproductive stages of the selected algae (Kim and Henriquez 1979; Yao et al. 1984; Marinho-Soriano et al. 1999; Mantri et al. 2009)

5. Conclusion

G. dura cultured for 25–35 days showed highest DGR with good quality agar properties and found suitable for commercial cultivation of *G. dura* along the Saurashtra coast of India. Minimal culture period maintained the vegetative phase of *G. dura* and reducing the maturity resulted higher biomass yield and DGR. Less days of culture period provided higher number of harvests cycles which may be beneficial for the cultivators to earn more profit from produced biomass. Therefore, the culture period of 25–35 days found ideal and suggested for commercial farming of *G. dura* along the west coast of India. The floating monoline is the simplest, time consuming and cost effective method compared to other methods; although, like other methods it also needs regular maintenance and cleaning of epiphytes.

Declarations

Acknowledgements:

Authors are highly thankful to Director, CSIR-CSMCRI, Bhavnagar and Co-DCs, Applied Phycology and Biotechnology Division, CSIR-CSMCRI for their support render during the study period. Authors are thankful to Dr. C. R. K. Reddy, ex-chief scientist, CSIR-CSMCRI, Bhavnagar for his significant initiative and support to get this project from NFDB, Hyderabad, Telangana, India. Authors are also thankful to National Fisheries Development board (NFDB) Hyderabad, Telangana, India & CSIR (HCP0024), India for providing financial assistance for this project. This contribution has CSIR-CSMCRI PRIS registration number CSIR-CSMCRI 11/2022.

Author contributions

Conceptualization, cultivation experiments, manuscript writing: Monica Gajanan Kavale, Review and Editing: M. Persis; Agar characterization: Ramavatar Meena; statistics, review and editing V. Veeragurunathan

Funding Acquisition: Monica Gajanan Kavale.

Funding

The work was supported by National Fisheries Development board (NFDB) Hyderabad, Telangana, India for project funding (File reference: 13104/NFDB/Seaweed/CSIR-CSMCRI/2017-18) & CSIR, India (HCP0024).

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest: The authors declare no competing interests.

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Figures

Figure 1

Map of study area

Figure 2

a. Floating monoline with initial seeded *G. dura*

b. Floating monoline ready for harvest

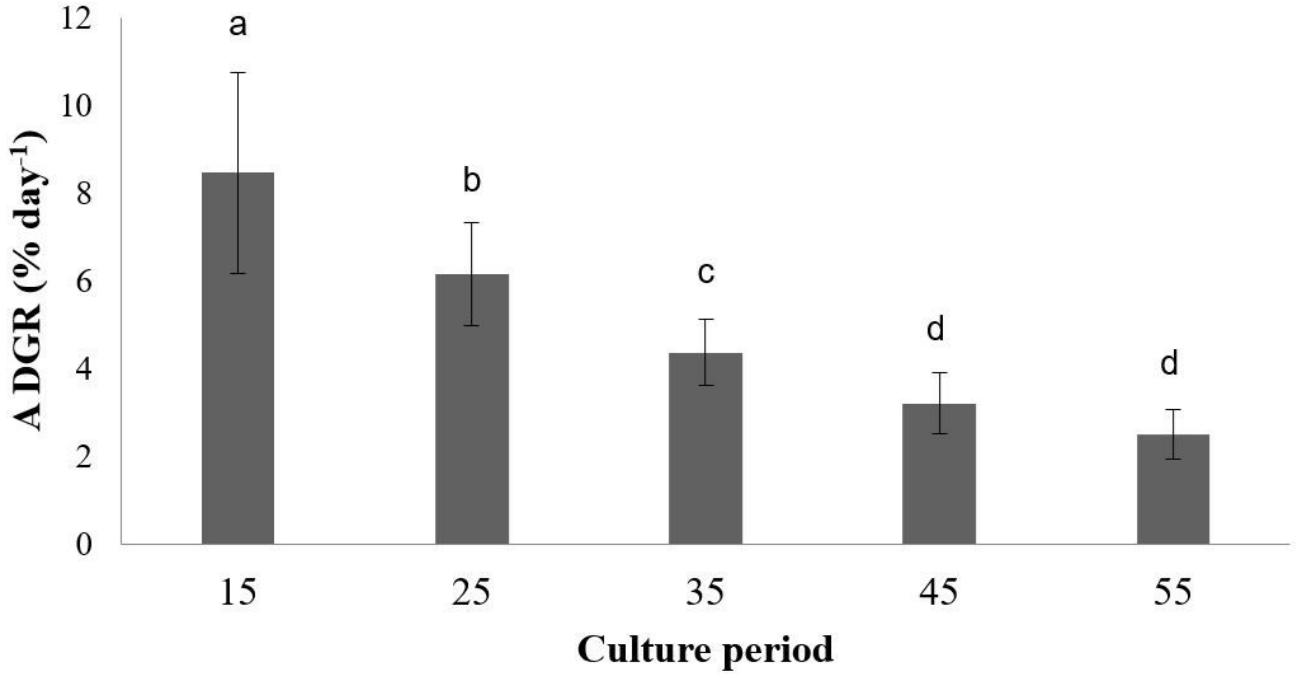

Figure 3

Average DGR (%) recorded in different culture period (15-55) for five harvest cycles (n=30). The small letters above the bar indicate the post ANOVA Tukey's test results for differences found in DGR in different culture periods ($p < 0.05$).

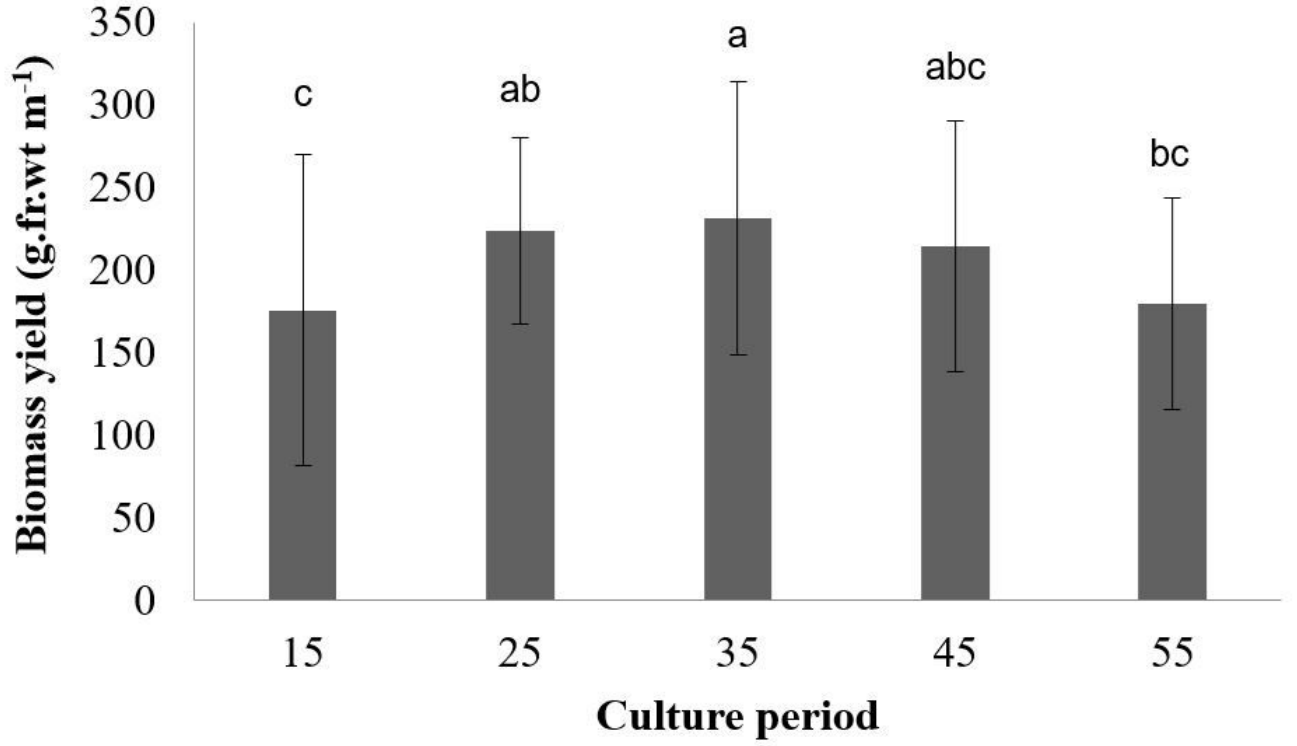

Figure 4

Average biomass yield (g fr.wt.m^{-1}) recorded in different culture period (15-55) for five harvest cycles (n=30). The small letters above the bar indicate the post ANOVA Tukey's test results for differences found in biomass yield in different culture periods ($p < 0.05$).

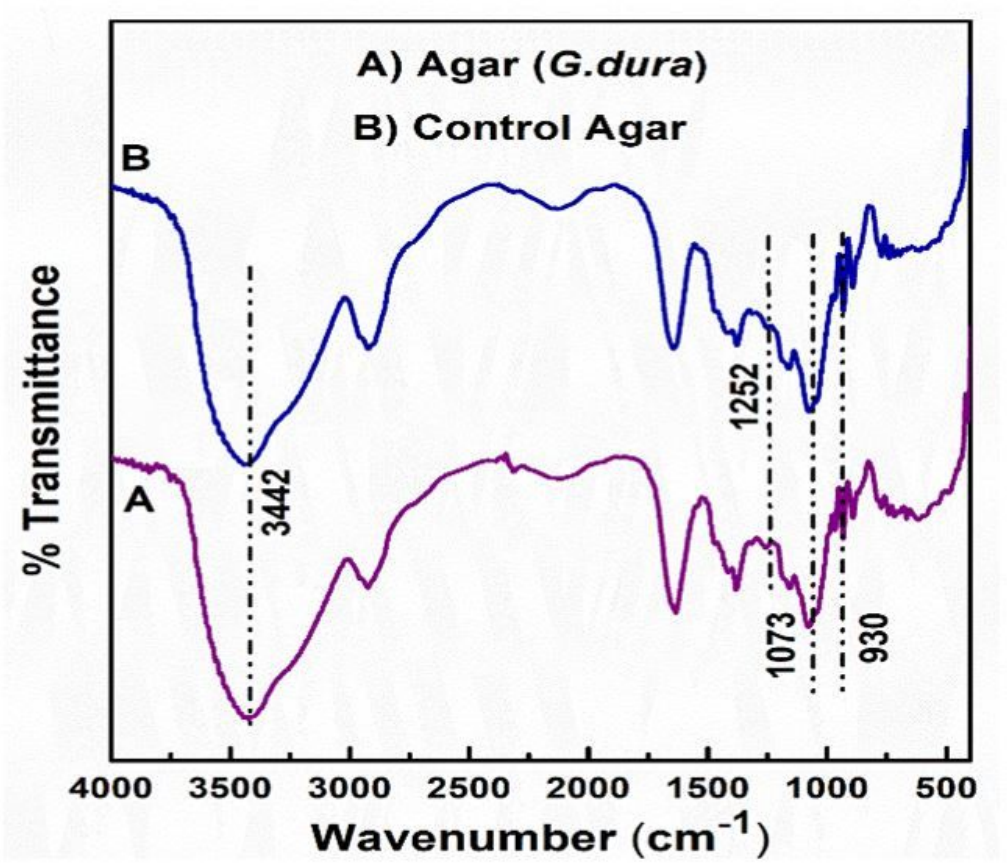

Figure 5

Shows FTIR spectra of (a) *G. dura* agar, and (b) control agar (Hi Media)