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Investigating the Effect of Different Growth Media on Biomass Production of *Pseudopediastrum boryanum* (Turpin) E. Hegewald Isolates

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ABSTRACT

Microalgae *Pseudopediastrum boryanum* (Turpin) E. Hegewald was chosen as a subject for the present research due to its potential uses of wastewater treatment and biodiesel production. In the present study, we investigated the growth and biomass production of *P. boryanum* through use of semi-continuous cultures employing two growth media (Allen and BG-11). In our previous study, *P. boryanum* was isolated from different freshwater reservoir through the dilution technique. The isolated *P. boryanum* strain was inoculated with 270 mL of medium + 30 mL of suspension culture and the 16:8 light/dark photoperiod was applied. Optical density was recorded by using UV-Visible spectrophotometer at 670 nm, and cell count examination was performed through drop count method. Besides, dry weight and chlorophyll-a concentration of strain were determined. The highest cell density ($3.67x10^6$ cells/mL), dry weight (0.032 g/mL) and chlorophyll-a (16.39 μgL^{-1}) production were observed in the Allen medium. Growth rates of *P. boryanum* were found to be 0.6676 d^{-1} in the Allen and 0.6021 d^{-1} in the BG-11 medium.

Keywords: *Pseudopediastrum boryanum*, optical density, cell density, growth parameters, culture conditions.

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Farklı Besi Ortamlarının *Pseudopediastrum boryanum* (Turpin) E. Hegewald İzolatlarının Biyokütle Üretimine Etkileri Üzerine Araştırma

Öz: Mikroalg, *Pseudopediastrum boryanum* (Turpin) E. Hegewald, atık su arıtımı ve biyodizel üretim potansiyelinden dolayı araştırma konusu olarak seçilmiştir. Bu araştırmada, iki besi ortamı (Allen ve BG-11) kullanılarak, kesikli kültür sisteminde *P. boryanum*'un büyüme ve biyokütle üretimi araştırılmıştır. Önceki çalışmalarımızda, *P. boryanum* dilüsyon tekniği kullanılarak, farklı tatlısu birikintilerinden izole edilmiştir. İzole edilen *P. boryanum* suşu, 270 mL besi ortamı + 30 mL süspansiyon kültür ile aşılanmış ve 16:8 aydınlık/karanlık fotoperiyodu uygulanmıştır. Optik yoğunluk 670 nm'de UV-Visible spektrofotometre kullanılarak tespit edilmiş ve suşların hücre sayımı damla sayım metodu kullanılarak yapılmıştır. Kültürlerin kuru ağırlık ve klorofil-a tayinleri de gerçekleştirilmiştir. Allen ortamında en yüksek hücre yoğunluğu ($3,67x10^6$ hücre/mL), kuru ağırlık (0,032 g/mL) ve klorofil-a (16,39 $\mu g\ L^{-1}$) olarak tespit edilmiştir. *P. boryanum*'un büyüme oranları Allen besi ortamında 0,6676 d^{-1} ve BG-11 ortamında 0,6021 d^{-1} olarak bulunmuştur.

Anahtar kelimeler: *Pseudopediastrum boryanum*, optik yoğunluk, hücre yoğunluğu, üreme parametreleri, kültür koşulları.

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Introduction

The recent developments in industrialization coupled with the increase in human population have led to exhaustion of the available natural resources within a short period of time. Besides, the increase in expectations in various industrial fields and the environmental pollution has paved the way for the research on the use of non-familiar resources for balanced and sufficient nutrients to feed the increasing population. As a solution to those problems, algae show itself as the light of hope (Sasson 1988). It is a known fact that algae contain rich nutrients; therefore, making them plays a vital role in the food chain. Along with the

use of biomass obtained from algae in food, cosmetics, chemistry, pharmacy etc., algae are also used in waste water treatment because of their ability to adhere to heavy metals (Spolaore et al. 2006). Also, as an alternative energy source, suitable algal species are used in the production of biodiesel (Brennan and Owende 2010).

Microalgae are single-cell, colony or filamentous forms of microorganisms convert solar energy into chemical energy through photosynthesis (Hosikian et al. 2010). While algae play a key role in the food chain, they convert inorganic molecules (such as carbon, nitrogen and phosphorus) into organic molecules in aquatic ecosystems (Murdock and Wetzel 2009). The microalgae are the complex heterogeneous microorganisms involving phylum with different physiological properties. As a result of that large diversity, different algae species have different growth requirements. The research into habitat of the microalgae revealed precisely that those microorganisms need certain nutrients. Along with the nutrients, temperature, intensity of light, the amount and type of nutrients, the amount of CO_2 and pH are primary factors affecting the growth of the algae (Kumar and Das 2012; Idenyi et al. 2016).

The genus *P. boryanum* is colony forms of the green algae occurring commonly in natural freshwater environments. Recently, it has become a topic of interest for the researchers. The algal species *P. boryanum* has beneficial attributes for wastewater treatment, particularly due to its high productivity and efficient removal by simple gravity sedimentation (Park et al. 2014). A pilot Cr (VI) biosorption study was carried out with the *P. boryanum* strain that we had isolated and used in the present study and the result was successful (Baykal Ozer et al. 2012). Microalgae *P. boryanum* was chosen as a subject for this research due to its potential uses of waste water treatment and biodiesel production. In this study, it is aimed to determine the characteristics of biomass production in different growth media of *P. boryanum*.

Material and Methods

Isolation

In our previous study, the samples, were collected from different freshwater reservoir, were brought to incubation at room temperature after inoculation at pre-enrichment nutrition media ($MgSO_4 \cdot 7H_2O$-2.50 g, KNO_3-5.0 g, KH_2PO_4-1.25 g, $FeSO_4 \cdot 7H_2O$-0.009 g and distilled water 1000 mL). *P. boryanum* was identified at species base with microscopic examination after incubation (Bourrelly 1972; Prescott 1975). From the mixed species in the pre-enrichment medium, *P. boryanum* was isolated through dilution technique (CSIRO 2017).

Culture Conditions

In this study, semi-continuous culture system was utilized in reproduction of the cultures. Allen and BG-11 media were used for experiments (Table 1). The isolated *P. boryanum* strain was inoculated with 270 ml of medium + 30 ml of suspension culture. The pH of nutrient media was adjusted as 6.5-7. The implementation of 16:8 light/dark photoperiod (50 µmol photnos $m^{-2}s^{-1}$) was applied on cultures and they were cultivated under at 22-25°C room temperature. All tests were carried out in triplets.

Determination of Cell Density

Total cell counts were microscopically determined through drop count method. In the course of cell count, a 0.03 mL sample was dropped on the slide and lamella was covered. When the microscope objective lens was at 10x40, 36 views were identified. The calculation was made by counting at least six views and proportioning into 36 transect views.

Determination of Dry Weight

The 50 mL samples were filtered via Whatman GF/C filter papers and dried at 60°C incubator and they were weighed (Chia et al. 2013). Dry weight determination was made on 14^{th} day following the cultivation process.

Chlorophyll-a Determination

The biomass of microalgae sample was estimated from their chlorophyll-a content measured through use of methanol method (Youngman 1978).

Maximum Absorbance Determination

The maximum absorbance was inspected by scanning sample cultures between 550 and 800 nm, using a UV–visible spectrophotometer (Biochrom Libra S22). The maximum absorbance value for microalgae was used to perform the growth curve by optical density (OD) (Santos-Ballardo et al. 2015). Optical density was recorded as 670 nm for *P. boryanum*. A linear regression equation was derived in order to describe the relationship between optical density and cell density.

Cell Growth Efficiency

By using the growth kinetics, specific growth rate and duplication time were calculated (Godoy-Hernández and Vázquez-Flota 2006). Specific

growth rate and duplication time are presented as Eq. (1 and 2):

$$\mu = \frac{\ln X2 - \ln X1}{t} \quad (1)$$

μ: Specific growth rate

X1 and X2 = Biomass concentration at $t1$ and $t2$

$$DT = \frac{ln2}{\mu} \quad (2)$$

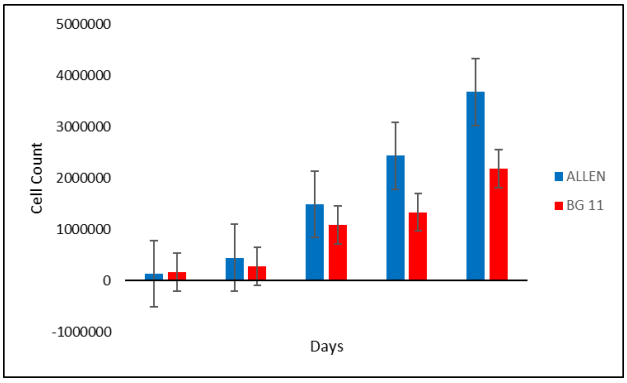
Table 1. Chemical compositon of the culture medium

Macroelements	Culture broth composition		
	BG-11 (g/L)	ALLEN (g/L)	(ml)
$NaNO_3$	1.5	1.5 g	
K_2HPO_4	0.04	-	
$K_2HPO_4 \cdot 7H_2O$	-	6 g/L	5 mL
$MgSO_4 \cdot 7H_2O$	0.075	6 g/L	5 mL
$CaCl_2 \cdot 2H_2O$	0.036	2.5 g/L	10 mL
Citric acid	0.006	4.8 g/L	1 mL
Ferric ammonium citrate	0.006	-	
EDTA (disodium salt)	0.001	-	
Na_2CO_3	0.02	4 g/L	5 mL
$Na_2SiO_3 \cdot 9H_2O$	-	4.64 g/L	10 mL
Trace metal mix A5	1.0 mL	-	
P-IV metal solution	-	1 mL	
Distilled water	1.0 L	200 mL	
Trace Metal mix A5		P-IV metal solution	
H_3BO_3	2.86	$Na_2EDTA \cdot 2H_2O$	0.75 g
$MnCl_2 \cdot 4H_2O$	1.81	$MnCl_2 \cdot 4H_2O$	0.041 g
$ZnSO_4 \cdot 7H_2O$	0.222	$ZnCl_2$	0.005 g
$NaMoO_4 \cdot 2H_2O$	0.39	$FeCl_3 \cdot 6H_2O$	0.097 g
$CuSO_4 \cdot 5H_2O$	0.079	$Na_2MoO_4 \cdot 2H_2O$	0.004 g
$Co(NO_3)_2 \cdot 6H_2O$	49.4	$CoCl_2 \cdot 6H_2O$	0.002 g
Distilled water	1.0 L	Distilled water	1.0 L

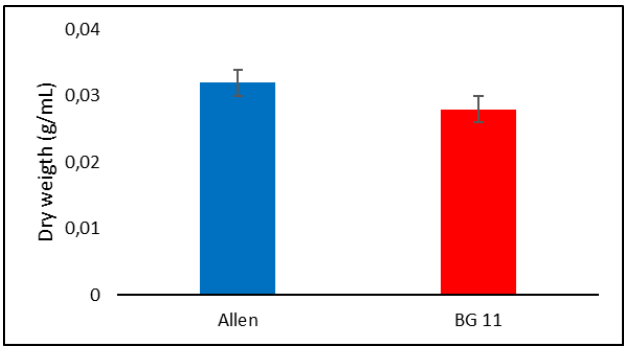
Results

The growth of microalgae *P. boryanum* in different culture media was primarily followed by counting algal cells under the microscope. Two growth media use for *P. boryanum* cultivation with varying chemical composition were studied. Both Allen and BG-11 medium were found to enhance the growth of *P. boryanum* at different densities. The biomass results obtained through two media of *P. boryanum* are presented in Fig.1. The highest cell density (3.67×10^6 cells/mL), dry weight (0.032 g/mL) and chlorophyll-a (16.39 µg L^{-1}) production were observed in the Allen medium (Fig. 1a, b and c)

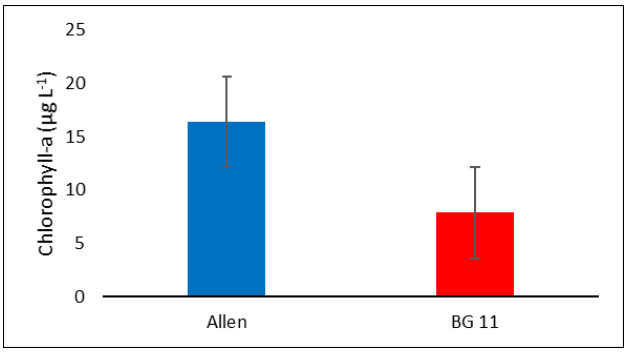
The calibrated data between optical density and cell density are displayed in Figure (2). The specific growth rate was calculated and presented in Figure (3). The duplication time was found as (0.0586) for Allen medium and (0.0577) for BG-11. Growth rates of *P. boryanum* were found to be 0.6676 d^{-1} in the Allen medium and 0.6021 d^{-1} in the BG-11 medium. Figure (4) indicates the effect of different medium on biomass productivity in ml/L/day. It is clear that the maximum biomass productivity was obtained in the Allen medium and the lowest in the BG-11 medium.



(a)



(b)

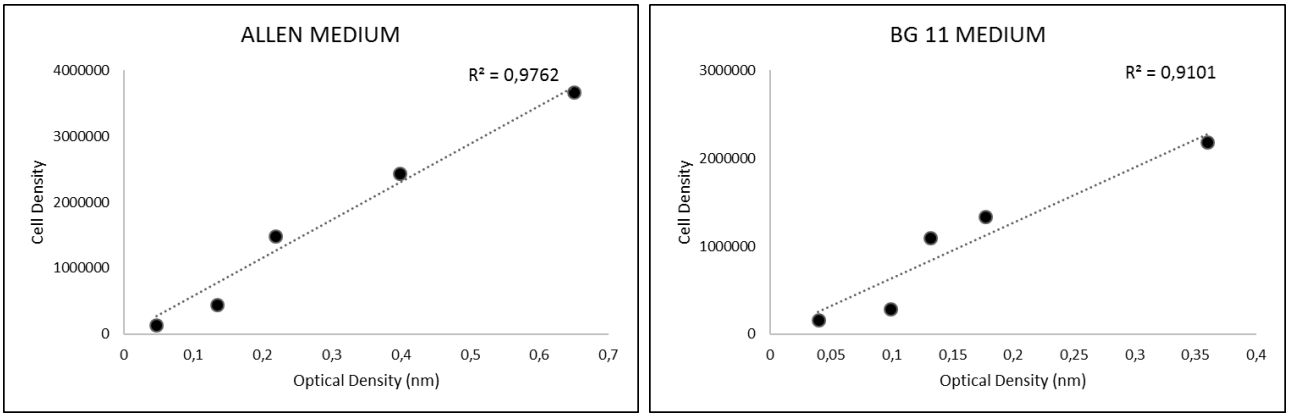


(c)

Figure 1. (a) Cell density (cells/mL); (b) dry weight (g/mL); (c) chlorophyll-a (µg L^{-1}) production. Error bars represent standard deviation for n=3.

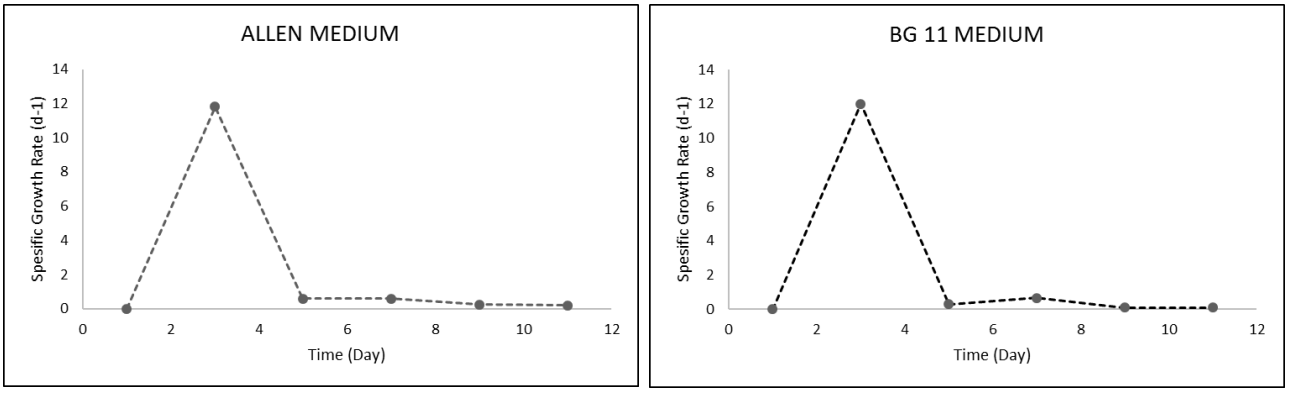
Discussion

There are differences among microalgae species in terms of cell growth and different microalgae groups have different physiological requirements in their natural habitats or under culture conditions (Falkowski 1984). Environmental and culture condition parameters such as light, photoperiod, medium and temperature influence the growth of microalgae (Ak et al. 2008; Fakhri et al. 2015).



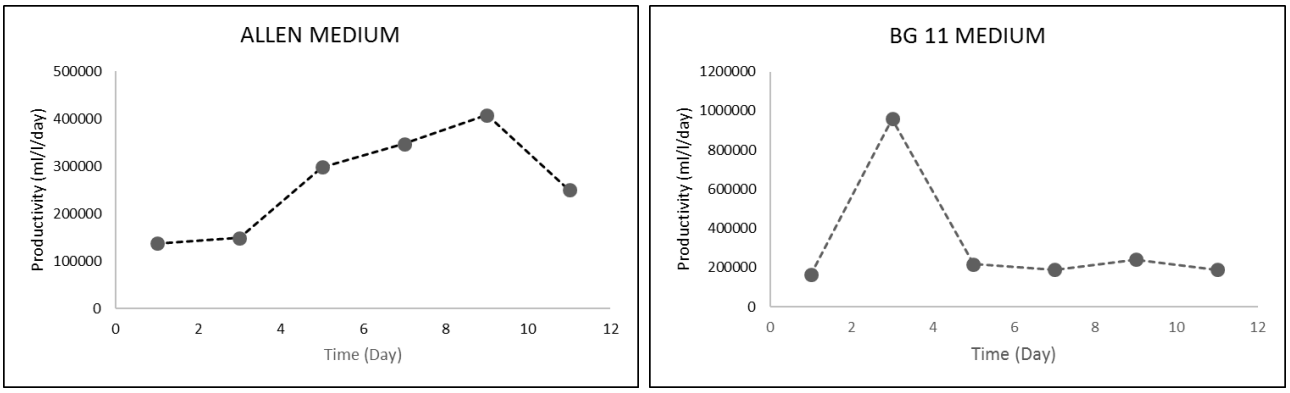
(a) (b)

Figure 2. Calibration curve for the relationship between optical density and cell density.



(a) (b)

Figure 3. Specific growth rate of *P. boryanum* microalgae at different nutrients.



(a) (b)

Figure 4. Biomass productivity at different media.

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The compositions and the amount of nutrients have great effect onreproduction of the microalgae. The lack of those substances may result in physiological and morphological changes in the microalgae. Especially, microalgae need the macronutrient elements (i.e. carbon, nitrogen, phosphorus), basic ions (i.e. Na^{+}, K^{+}, Mg^{2+}, $Ca2^{+}$, Cl^{-}, $SO4^{2-}$) and micronutrient metals (i.e. iron, manganese, zinc, cobalt, copper, molybdenum, nickel and cadmium) in their habitats (Duygu Yalcin 2017).

Phosphorus is the most needed macronutrient by the microalgae after nitrogen. Phosphorus is necessary for many phosphorylation syntheses and the Calvin cycle. Therefore, the lack of phosphorus affects not only the synthesis of chlorophyll but also

the growth and metabolism of cells (Liang et al. 2013). The KH_2PO_4 and K_2HPO_4 are the sources of phosphate for algal growth in the present study. Nitrogen is a significant substance for synthesis of protein, nucleic acids and chlorophyll molecules (Lourenço et al. 2004). $NaNO_3$ and EDTA are the nitrogen sources of both media which were used in this study. The magnesium plays a significant role in the growth of microalgae as a cofactor of some key enzymes in the metabolic pathway (Esakkimuthu et al. 2016). Magnesium source of growth media used in this study was $MgSO_4$. $MgSO_4$. was used in this study as Magnesium source of growth media. Iron is a vital element in algal growth and ferric ammonium citrate and $FeCl_3$ in the composition of the nutrients serving as the iron source for the culture in this study.

A series of measurements were performed in order to estimate growth rate and to calculate the rate of change occurring in biomass concentration. The cell number was determined through use of light microscope and the linear correlation between those measurements and cell number was determined through optical density.

Both Allen and BG-11 growth media use for *P. boryanum* cultivation with different chemical compositions were studied. Cell density which is an important biomass parameter varied significantly from medium to medium. The present study mainly dealt with the evaluation of growth rate (cell density) of *P. boryanum* which showed variations in their growth pattern in the two-growth media. The growth of *P. boryanum* in different culture media was evaluated by microscopic cell counting value. In general, Allen Medium was found to greatly influence the growth of *P. boryanum* than BG-11 medium. Based on the density concentration measurement, it was observed that the growth of *P. boryanum* was highly favoured by Allen Medium (Figure 1a). For (n=3), the cell density was found to be ($3.67x10^6$ cells/mL) in Allen Medium and ($2.19x10^6$ cells/mL) in BG-11 Medium at the end of 11th day. The results of this study are similar to those of Park et al. (2014) investigating the growth of *P. boryanum* under controlled conditions. The dry weight and the amount of chlorophyll-a were measured as (0.032 g/mL) and (16.39 µg L^{-1}), respectively in Allen Medium and they were found as (0.028 g/mL) and (7.854 µg L^{-1}) in BG-11 Medium in paralel with the cell number (Fig. 1b and 1c).

The optical density is an indirect method which is commonly used while measuring biomass of the microalgae, observing and controlling their growth. Through that method, the cell number can be correlated and adapted easily to the automatic measurement systems (Ribeiro-Rodrigues et al. 2011). In general, 660-690 nm is suggested for standard tests carried out while measuring microalgal growth by spectrometer (Bricaud et al. 1998). Wavelength was scanned from 550 to 800 nm and maximum absorbance was observed at 670 nm for the analysed microalgae in different media. A linear dependence between absorbance and cell counting is assumed when absorbance is measured at the same wavelength (670 nm) for *P. boryanum* (Santos-Ballardo et al. 2015). Growth performance was influenced by media type, which supports higher growth rate (0.6676 d^{-1}) for *P. boryanum* in the Allen medium in comparison with that (0.6021 d^{-1}) in BG-11 culture media tested. That growth rate is among the values stated in the literature for *P. boryanum* (Park and Craggs 2011; Park et al. 2013). Park et al. (2014) found out the maximum growth rate of colony (h^{-1}) 0.097±0.023 at 20°C and (h^{-1}) 0.060±0.002 at 10°C. In that study, a very good positive correlation in both culture media was obtained for *P. boryanum* ($r_=$0.9762 and $r_=$0.9101, respectively). The fastest doubling time resulting from *P. boryanum* was Allen medium (0.0586) and BG-11 (0.0577), respectively.

Al-Shatri et al. (2014) carried out a study by evaluating the effects of different algal nutrient medium constituents in order to obtain cell number of optimised *Scenedesmus dimorphus.* They stated that they could obtain the highest yield through BG-11 nutrient media after Bold's Basal Medium (BBM). Besides, a study was conducted to determine the most suitable nutrient media with the aim of obtaining high biomass production rate of *Chlorella minutissima* (Singh et al. 2014). Growth measurements revealed that BG-11 medium enhanced biomass production. In the study conducted upon *Lyngbya bipunctata*, the effect of different nutrient medium was examined and it was found that the nutrient medium of BG-11 and Allen increased carotenoid amount with their wet and dry weight (Nehul 2014). The studies conducted upon different microalgae species revealed that BG-11 and Allen nutrient medium were effective in culturing of the microalgae.

The temperature in culture media in the present study was determined to be between 22-25°C and *P. boryanum* cultures showed good development under that temperature. The light is the energy source of photosynthesis and a necessary factor to transform inorganic carbon into organic molecules and for growth and to obtain energy. Therefore, the cultures were applied 16:8 h light/dark cycles (Falkowski 1984; Wahidin et al. 2013).

In summary, this study mainly focused upon the effect of Allen Medium and BG-11 Medium on the

growth of fresh water microalgae, that is, *P. boryanum*. It was clearly observed that Allen Medium had a greater influence on the growth of *P. boryanum* when compared with BG-11 medium.

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