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## **COVERING LETTER**

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I herewith enclosed a research article,

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Characterization of Arthrospira cultured in waste water of Clarias catfish farming media : DNA barcode, helical form, growth, and phycocyanin

## Author(s) name:

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Characterization Arthrospira cultured in Clarias waste water based on DNA barcode, helical form, growth, and phycocyanin vs. Arthrospira cultured in general media (fertilizer, Zarrouk media, Aiba Ogawa media, Walne)

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## Place and date:

Palembang, Indonesia, 31 October 2020

## Sincerely yours,

(fill in your name, no need scanned autograph) Marini Wijayanti

# Characterization of Arthrospira cultured in waste water of Clarias catfish farming media: DNA barcode, helical form, growth, and phycocyanin

### MARINI WIJAYANTI<sup>VI</sup>, M. SYAIFUDIN<sup>1</sup>, YULISMAN<sup>1</sup>, YULLY NURIANTI<sup>1</sup>, ANITA HIDAYANI<sup>1</sup>, NUNI GOFAR<sup>2</sup>

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Abstract. Arthrospira production technology in catfish waste media can be an alternative to reduce environmental pollution. However, some environmental factors such as nutrition, light and water content can influence characterization of Arthrospira at the genetic and physiologic level. Arthrospira platensis is one of the phycocyanin-producing cyanobacteria and can be cultured using catfish culture wastewater. Water quality especially pH and salinity can effect of growth rate and rendement of phycocyanin from Arthrospira platensis. This study aimed to identify the species and morphological forms of Arthrospira cultured using technical fertilizer and waste media, as well as to know the phylogenetic trees between species in this study and the GeneBank based on the 16S rRNA gene, and determine the optimum of pH and salinity required in the medium of catfish culture wastewater to phycocyanin maximum production of Arthrospira. The optimation of pH and salinity method used Completely Randomized Design (CRD) factorial with 2 factors consisting of the first factor with 3 treatments and the second factor with 4 treatments and 3 replications. The first factor was pH of culture medium i.e. pH  $6.5 \pm 0.2$ , pH  $8.5 \pm 0.2$  and pH  $10.5 \pm 0.2$ . The second factor was salinity of culture medium, that were salinity 0 ppt, 10 ppt, 20 ppt and 30 ppt. Parameters observed in Arthrospira include density, growth rate, rendement of phycocyanin, and decreased of total nitrogen and phosphate content in culture media. The results showed that morphology Arthrospira cultured on technical fertilizer media (AF) had a longer and helix filament compared to Arthrospira cultured on waste media (AW) which showed several linear and shorter filaments. Both samples have a genetic distance of 0.068 (6.8%). Phylogenetic trees indicated that AF had a close relationship with Arthrospira platensis petH from Japan (bootstrap value 95%). While AW formed a separate sub cluster of AF isolates and Arthrospira platensis petH from Japan (bootstrap value of 85%). The best treatment in this study was P2S3 (pH  $8.5 \pm 0.2$  with salinity 20 ppt), which produced 0.867 grams maximum density, growth rate of 22.026 % day<sup>-1</sup> and 11.347 mg.g<sup>-1</sup> rendement of phycocyanin.

Key words: catfish culture wastewater, DNA barcode, pH, phycocyanin, phylogenetic analysis, salinity, *Spirulina(Arthrospira)*,16S rRNA

Abbreviations:  $AF = Arthrospira \ platensis$  cultured on technical fertilizer media;  $AW = Arthrospira \ platensis$  cultured on waste media

**Running title:** a short title with five words

## INTRODUCTION

*Arthrospira* is a genus of cyanobacterial microalgae, commonly known under the taxonomically incorrect brand name 'Spirulina' (Papapanagiotou & Gkelis, 2019). The cyanobacterial genus Arthrospira Stizenberger ex Gomont 1892 contains at present 23 species, along with 12 infraspecific taxa (Guiry & Guiry, 2018). They have variety characteristic of molecular, morphology, and physiology that based on polyphasic approach. Various genotypes are adaptable to various specialized ecosystems. The combination of different methods should be based on molecular sequencing as the basic approach, to which must be added other criteria (morphological, ecological) if they are available and which are distinct and recognizable in cyanobacterial populations (Komarek, 2016). A polyphasic approach to include all the criteria obtained from morphological, biochemical, molecular studies, and phylogenetic to understand cyanobacterial classification as like as *Arthrospira* classification (Komárek, 2018).

Recent studies have shown that Arthrospira can be used for treating wastewater, including effluent from fish culture, because the biomass can metabolize the nutrients and remove the polutant from aquaculture effluent efficiently (Zhang et al., 2020). Industrial and processing wastes and by-products for culturing Spirulina (*Arthrospira*) are also being considered as alternative culture media, as like as aquaculture waste water (Ragaza et al. 2020, Wijayanti et al. 2018, Widyantoro et al., 2018). Aquaculture could apply an integrated strategy of

simultaneously treating aquaculture effluent while producing the biomass to supplement fish diets. The nutrient composition in their biomass depends on their environmental factor for growing biomass. Their character could be different with the various media for growth.

Basically, *Arthrospira*'s morphology is characterized by trichomes that circular regularly (helical). However, abnormal morphology can also occur in *Arthrospira* as a circular shape that is irregular even linear. In some cultivation conditions, linear filaments can spontaneously return to the helix. However, there are significant differences in morphology, ultrastructure, physiology, biochemistry, and genetic characteristics between the original filament and the linear filament but not the difference between the original and the returned filament. Linearization in *Arthrospira* is a variation on the genetic level that can be caused by several environmental factors such as nutrition, light and content of water media for growth (Wang and Zhao 2005). According to Liu et al., (2016), DNA barcoding has developed as a reliable technology for identifying species based on variations in the sequence of standard DNA regions. This method is used successfully in a variety of biological applications including finding cryptic species, detecting invasive species, and identifying plants. DNA barcoding is a simple short genome sequence amplified via PCR using appropriate primers (Adamowicz, 2015). DNA barcoding using the 16S rRNA gene needs to be done to get the characterization of *Arthrospira* that is cultured on technical fertilizer and waste media and determine the phylogenetic tree structure that has been recorded in GenBank.

Culture of Arthrospira (Spirulina) in Clarias pond farming wasted water could have specific characterization for optimal pH value and salinity. Their adaptation to grow in organic waste water make change in bioactive and important compound production. Their biomass has a nutritional value of 55-70% protein, 6-10% lipid, 20% carbohydrate, besides being rich in minerals, vitamins, and pigments (Borowitzka et al., 2016; Vernes et al., 2016). Some color pigments that can be produced such as phycocyanin (blue pigment), allophycocyanin (blue-green) and phycoerythrin (red pigment) (Sharma and Tiwari, 2011; Vernes et al., 2015). Phycocyanin is pigment in Arthrospira which has functions as an antioxidant (Pirenantyo and Limantara., 2008), a source of food coloring, cosmetics, pharmaceuticals and drugs (Tang et al,. 2020; Tiwari & Tiwari, 2020), antiinflammatory, anti-oxidative and anticancer (Liu et al., 2013). One of the factors that influence phycocyanin levels is biomass (Taufiqurrahmi et al., 2017). The pH and salinity of culture media can affect the biomass of Arthrospira (Ciferri, 1983; Marek et al., 1987; Planes et al., 2002). Ismaiel et al. (2016) showed that the diversity of the chemical composition of biomass is influenced by the pH of the growth media. Value of pH and environmental factors, especially salinity, influence the productivity of cell biomass, photosynthesis, shape, and flow of cellular metabolic activity that affect the dynamics of cell composition (Hu, 2004). The optimal pH value for growth of Arthrospira sp is 7-10.5 (Hariyati, 2008), and salinity from 15-30 ‰ (Thajuddin and Subramanian, 2005). The salinity and pH value of Arthrospira culture media have been known to affect the morphology of the filament.

The aims of this study is characterizing morphological forms and DNA barcode based on the 16S rRNA gene of *Arthrospira* (*Spirulina*) cultured in fertilizer and waste water effluent of *Clarias* pond farming media, and determining optimal pH value and salinity of culture media for growth and phycocyanin production, especially in *Clarias* pond farming waste water media and morphological changes of their filament.

### MATERIALS AND METHODS

## Arthrospira cultured in agar media

Bacto agar was weighed as much as 2 g dissolved in 100 ml of water. The water used was swamp water and catfish culture waste that has been filtered and sterilized using an autoclave. Sterilized swamp water was then added with 0.02 gram MgSO4 fertilizer; CaCl<sub>2</sub> 0.004 gram; EDTA 0.008 gram; urea 0.03 gram; ZA 0.132 grams; 0.4 gram baking soda; AB solution 1 ml (A solution 20 grams / 100 ml B solution 20 grams/100 ml water) and TSP 0.05 grams were then homogeneous using magnetic stirrers. Next, wasted water was sterilized by an autoclave then cooled. Bactoagar was added to the technical fertilizer and waste solution to be homogenized using a magnetic stirrer and then boiled using a hot plate until all the ingredients dissolve and then autoclave again. The agar media was made with a pH of 7 and a salinity of 10 g.L<sup>-1</sup>.

Arthrospira cultivated in liquid media was taken 100  $\mu$ l using micropipette and spread to the surface of a petri dish containing bactoagar media by using a sterilized spreader rod. Petri dishes were wrapped in wrapping plastic and then given a lamp lighting (light intensity 2000-4000 lux) with a dark: light ratio = 0:24 hours.

*Arthrospira* was observed every day until it grows blue green. After growing, *Arthrospira* was re-cultured in agar media by the 4 quadrant scratch method. The cultures were used as isolate samples for determining DNA barcodes.

### DNA extraction

DNA extraction was carried out according to procedures in which there was a Presto TM Mini gDNA Bacteria Kit (Geneaid Biotech Ltd.). DNA extraction consisted of several stages: sample preparation, lysis, purification, and precipitation or washing. The sample used was 0.15 grams of wet weight for one extraction.

## **DNA** Amplification

The process of DNA amplification using the PCR (Polymerase Chain Reaction) method was performed using 2  $\mu$ l forward primers 63f (5'-CAGGCC TAA CAC ATG CAA GTC-3 ') and reverse primer 1387r (5'-GGG CGG WGT GTA CAA GGC-3 ') (Marchesi et al., 1998). The total composition of the PCR mixture was 50  $\mu$ l which consisted of 25  $\mu$ l Go Taq Green, 13  $\mu$ l NFW (Nuclease Free Water) and 8  $\mu$ l *Arthrospira* DNA extraction template. DNA amplification was carried out in stages: the initiation cycle at 95°C for 5 minutes, followed by 30 denaturation cycles at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, then the extension stage at 72 °C for 1 minute, and the final stage 72 °C for 7 minutes (Lee et al., 2003).

### Electrophoresis

Electrophoresis was carried out using 1% agarose gel at 75 V for 35 minutes. Agarose that have been electrophoretic was immersed with a mixture of 10  $\mu$ l diamond dye solution and 100 ml TAE 1x buffer solution for 30 minutes without exposure to light. The results were visualized through gel documentation by observing DNA migration using a transiluminator UV.

### Gene Sequencing

*Arthrospira* DNA samples that were successfully amplified using PCR were then sequenced in the fragments of 16S rRNA gene. The amplified products were sequenced through the services of the Macrogen Institute in Jakarta. The *DNA* sequences obtained in the form of fasta format were aligned using MEGA 6.0 software and then uploaded through the Basic Local Alignment Search Tool (BLAST) program. BLAST was a program to search for and analyze the homology of an organism's sequence, on the ncbi.nlm.nih.gov website so that its homology can be identified with other genus *Arthrospira* 16S rRNA gene sequences registered in the GenBank database. The genetic distance and phylogenetic trees between genera were constructed using the Neighbor Joining (NJ) method. The phylogenetic tree was constructed through the Mega 6.0 software application using the Neighbor-Joining (NJ) method of the Maximum Composite Likelihood model and Substitutions to include d: Transitions + Transversions with 1000x boostrap. Meanwhile *Arthrospira* to the appropriate literature.

### Optimization of pH and salinity for growing Arthrospira in Catfish farming wasted water

The experimental design for optimizing pH and salinity media for growing Arthrospira in Catfish farming wasted water was a Factorial Completely Randomized Design (CRD) consisting of the first factor with 3 treatments and the second factor with 4 treatments and 3 replications. The first factor was the difference of pH in culture media, including P1: culture media pH  $6.5 \pm 0.2$ , P2: culture media pH  $8.5 \pm 0.2$  and P3: culture media pH  $10.5 \pm 0.2$ . The second factor was the difference of salinity in culture media i.e. S1: salinity of 0 ‰, S2: salinity of 10 ‰, S3: salinity of 20 ‰, and S4: salinity of 30 ‰.

### Culture preparation

The equipment used in this study was sterilized using 70% alcohol to minimize the contaminants that inhibit the productivity of *Arthrospira*. The containers used plastic bottles with capacity of 5 L volume of 36 units. The plastic bottle were sterilized using a potassium permanganate solution (2 mg. L<sup>-1</sup>). Catfish culture wastewater obtained from catfish farming ponds measuring 2 m x 1 m x 1 m, and high of water media was 20 cm (Figure 1). The density used in the pond was 330 fish.400 L<sup>-1</sup> with 150 grams fish<sup>-1</sup>, maintained for 2 months by providing artificial feed (protein 31%-33%), twice per day at satiation. Catfish culture wastewater was previously sterilized by boiling in an autoclave and then cooled, while the steril wastewater was treated with salinity. In treatments S1, S2, S3 and S4 were added with salt until salinity was obtained according to the treatment. The wastewater media had a pH of 7.3, therefore there was an addition of HCl 1 N of 0.75 ml L<sup>-1</sup> in P1 treatment to reach a pH of 6.5. Meanwhile, in treatments P2 and P3, to get a pH of 8.5 and pH 10.5 there was an addition of NaOH 8 N as much as 0.07 ml L<sup>-1</sup> and 0.45 ml L<sup>-1</sup>.



Figure 1. Catfish farming pond



Figure 2. Arthrospira cultivation

## Arthrospira cultivation.

*Arthrospira* previously used was cultured in catfish culture wastewater for culture stock with a density of 2 g L<sup>-1</sup>. The stock was taken as much as 400 ml in 3600 ml of catfish culture wastewater in accordance with treatment. Aeration was used for agitation, the lighting using 36 watt TL lamps for 24 hours day<sup>-1</sup> during maintenance (Figure 2).

Harvest of biomass.

Harvest of the biomass was after exponential phase by filtering. The biomass was dried using an oven for 14 hours at 40  $^{\circ}$ C (Afriani et al., 2018 with modification). The dry biomass was used for the phycocyanin extraction process.

## Phycocyanin extraction

The dry biomass was 0.04 g added by 1 ml of phosphate buffer pH 7, then homogenized and frozen in the freezer for 24 hours at a temperature of -4 °C. After 24 hours from the freezer, thawing process for 15 minutes. Samples were centrifuged for 30 minutes at 3000 rpm. After that, the sediment and the supernatant were separated. The resulting supernatant was phycocyanin which be analyzed using the Bennett and Bogorad method (1973).

## The density of Arthrospira biomass.

Biomass density measurements were performed at each treatment and 3 replications every day at the same hour. The density of biomass was 1 ml of sample in each treatment with 3 replications. The 1 ml of sample into aluminum bowl. The sample and the aluminum bowl were weighed, then dried in the oven for 14 hours at 40  $^{\circ}$ C. The sample of water that had dried was weighed again. The dry biomass weight of *Arthrospira* biomass was converted to g L<sup>-1</sup>.



Figure 3. Dry biomass of Arthrospira platensis after oven

The growth rate of Arthrospira can be calculated using the following formula according to Vonshak (1997):

$$\mu = \frac{\ln Nt - \ln No}{t} \times 100\%$$

Note :  $\mu$  = daily growth rate (% days<sup>-1</sup>) t = time (days) from N<sub>0</sub> to N<sub>t</sub> N<sub>0</sub> = initial density (g L<sup>-1</sup>)

 $N_0 =$  initial density (g L<sup>-1</sup>)  $N_t =$  density at the time t (g L<sup>-1</sup>) Measurement of total nitrogen and phosphate content in culture media was carried out at the beginning and the first day after the peak phase of each treatment.

The measurement of photocyanin refers to Bennett and Bogorad (1973). The absorbed supernatant was measured using a spectrophotometer at wavelengths of 615 nm and 652 nm.

C-phycocyanin (mg.mL<sup>-1</sup>) = 
$$(OD \ 615) - 0.474 \ (OD \ 652) = 5.34$$

Rendement percentage of phycocyanin (%) = Rendement of phycocyanin (mg  $g^{-1}$ ) x 100%

Note : C-phycocyanin = C-phycocyanin consentration (mg. mL<sup>-1</sup>) V = Solvent Volume (ml) DB = Dry Biomass (0.04 g) 0.474 and 5.34 = coefficient of extinction (Bennett and Bogorad, 1973)

The results were submitted to simple analysis of variance tests (ANOVA) (p < 0.05) and in the case of significant differences, the means were compared by the Least Significant Differences test (p < 0.05).

## **RESULTS AND DISCUSSION**

### Morphology of Arthrospira

Arthrospira was cultivated using two different fertilizer media namely technical fertilizer and waste media. The morphology of commercial Arthrospira before fertilizer treatment was presented in Figure 4.



Figure 4. Morphological identification results of *Arthrospira* isolate (CA =Commercial *Arthrospira*; AF= commercial *Arthrospira* cultured with technical fertilizer media; AW= commercial *Arthrospira* cultured with waste media) 40x magnification

The results of the identification of isolates showed that the isolate had a twisted filament shape resembling a spiral (helical). Based on Davis's identification book (1955), it is known that the isolate used in the study was *Spirulina (Arthrospira) platensis. Arthrospira* is cyanobacteria belonging to the order Oscillatoriales which has a filament (trichome) that resembles a spiral (helical) but does not have heterocyst cells (Sze, 1998). Heterocyst cells are special thick-walled cells that play a role in nitrogen fixation from the air (Fogg et al., 1979). In this study *Arthrospira* cultured in different media had several linear/straight morphologies.

Based on Figure 2, *Arthrospira* which was cultured on technical fertilizer media has a longer and spiraling morphological form compared to another cultured on waste media. Their filaments have more linear morphological form, some spirals but not too long. According to Astiani et al (2016), *Arthrospira* growth is influenced by nutritional and environmental factors. Wang and Zhao (2005) explained that linearization that occurs in *Arthrospira* is a variation on the genetic level that can be caused by environmental factors such as lack of nutrition and high light intensity. In this study, isolates were cultured with the same light intensity of 2000-3000 lux with a light dark ratio of 0:24 hours.. Linear filaments in AW have a lower metabolic rate compared to

helical filaments. This is one of the adaptive mechanisms for *Arthrospira* to survive some environmental conditions that are not appropriate.

Description	Identity (%)	Access code	Sample origin
Arthrospira (fertilizer)			
Arthrospira platensis petH	100	AB113346	Japan
Spirulina platensis CCC 478	90,48	JX014313.1	India
Spirulina platensis cyaG	94,4	D49531.1	Japan
Arthrospira platensis PCC 7345	90,12	JN831264.1	USA
Arthrospira maxima EEW2	74,4	HQ008225	Australia
Arthrospira (waste media)			
Arthrospira platensis petH	94,3	D49531.1	Japan
Arthrospira platensis DKCAS2	81,4	MG912588.1	India
Spirulina platensis CCC 478	74,4	JX014313.1	India
Arthrospira maxima str. Lefevre 1963/M-132-1	73,3	FJ798612	Venezuella
Arthrospira maxima EEW2	72,2	HQ008225	Australia

 Tabel. 1. The results of the BLASTn analysis of Arthrospira samples cultivated in technical fertilizer and waste media with data in Genbank

## **Phylogenetic Tree**

The results of the 16S rRNA encoding gene sequences from AF and AW isolates were traced to other *Arthrospira* isolates present in GenBank through the BLAST program. The results of the BLASTn analysis of *Arthrospira* samples cultivated in technical fertilizer and waste media with data in Genbank are presented in Table 1. Table 1 results of the BLAST analysis show the closeness between AF and AW isolates with other isolates in GenBank. It show that *Arthrospira* technical fertilizer isolates and *Arthrospira* waste isolates have the closest homology to *Arthrospira platensis* petH species from Japan with percentage values respectively 100% and 94.3%.



**Figure 5.** Phylogenetic analysis with 1000 bootstrap AW (*Arthrospira* cultured in waste water media) and AF (*Arthrospira* cultured in fertilizer media)

Genetic distance was used to see kinship relationships from *Arthrospira* both AF and AW samples with sequential data from Genbank. AF isolate indicated a genetic distance of 0.068 with AW isolates. AW and AF isolates showed the lowest genetic distance respectively 0.089 and 0.060 with *Arthrospira platensis* petH from Japan. Analysis based on genetic distance showed that both isolates were belong to the same species namely *Spirulina platensis*, however the genetic distance was 0.068 (6.8%) meaning that there are intraspecies variations in the sample caused by mutations.

Phylogenetic tree *Arthrospira* isolates from technical fertilizer and waste media were presented in Figure 5. The phylogenetic tree is a two-dimensional graph showing relationships between organisms or population classifications based on their evolutionary history. The result of phylogenetic tree construction showed that both samples formed branches with a cluster. Phylogenetic tree from AF and AW isolate sequences formed cluster was separate with several other *Arthrospira* species from GenBank data.

The AF isolates had a close relationship with *Arthrospira platensis* petH species from Japan with a bootstrap value of 95%. Hadiati (2003) states that bootstrap analysis is performed to determine the level of confidence in grouping. Bootstrap value is considered high because according to Hall (2001), a clade can be trusted with a bootsratp value of 90%. In addition, Hillis and Bull (1993) state that bootstrap analysis with values of 70% or higher indicate a reliable grouping. The AW isolates formed a separate brach of AF isolates and

*Arthrospira platensis* petH species. Genetically, they had diverse, and adapted to environmental conditions. The AW isolate indicated different strain from AF isolate groups. Ballot et al. (2004) stated that *Spirulina* from the same species and cultured under different conditions can form a separate subcluster with a bootstrap value of 79%. Zhao et al. (2006) identified and analyzed the number of restriction-modification genes in the cyanobacterial genome, seeing that more restriction-modification genes were found in cyanobacterial filaments (*Anabaena, Spirulina* and *Nostoc*) than dispenses (*Synechocystis, Synechococcus* and *Prochlorococcus*) this was due to the organism adapting to various environmental conditions, or the many variations in sources of nutrients that cause mutations.

Density and Growth Rate of Arthrospira cultured in wastewater of catfish farming

The biomass of Arthrospira displayed mechanism of adaptation in culture media. The wastewater media could make different charateristic of growth as like as filament. The maximum densities of *Arthrospira* cultured in wasted water were achieved on a different day. The daily density of *Arthrospira* during culture can be seen in Figure 6. Biomass density  $(g.L^{-1})$ 



Figure 6. Cell density (dry weight with a moisture content of 1.2%) of *Arthrospira* in catfish rearing pond waste water

The graph presented in Figure 6, it show that in the culture period from day 1 to day 2, *Arthrospira* in each treatment experienced slow growth, because the cells were still adapting to their new environment. The exponential phase for the treatment of P2S2, P2S3, and P3S3 last from day 1 to day 8 of the culture period. In the treatment of P2S1, P2S4, P3S1 and P3S2 take place from  $2^{nd}$  until  $8^{th}$  day of the culture period. While the treatment of P1S1, P1S2, P1S3, P1S4, and P3S4 lasted from  $3^{rd} - 8^{th}$  day of the culture period. The decreasing *Arthrospira* density for the treatment occurred from the day 9 to the day 11 of the culture. Lesmana et al. (2019) explained that the adaptation phase lasts from day 7 to day 9 then enters the death phase after the 7<sup>th</sup> day and 9<sup>th</sup> day. The decrease of density could because of reducing the nutrients in the culture media. Soni et al. (2019), the concentration of nutrients in the media decreased after reaching the peak period. This is due to the higher density of *Arthrospira* in the culture media.

The maximum density of *Arthrospira* could be achieved on different day, between 5 - 8 days after culturing. The mean of maximum density could be 0.433-0.867 g L<sup>-1</sup> of dry biomass which cultured in catfish rearing waste water. The maximum cellular density of *A. platensis* which cultured in nile fish rearing waste water, resulted in the production of 0.22 g L<sup>-1</sup> of dry biomass and maximum productivity of 0.03 g L<sup>-1</sup> day<sup>-1</sup> (Nogueira et al, 2018). The catfish rearing pond waste water has high potential as cultivation media for Arthrospira production.

The analysis of variance showed that differences in pH, salinity and interaction between factors (pH and salinity) significantly affect the maximum density and growth rate of *Arthrospira platensis*. The results of the LSD  $_{0.05}$  maximum density test and growth rate sequentially were presented in Table 1 and Table 2. LSD  $_{0.05}$  test results on the main factors of differences in pH, density and growth rate of *Arthrospira platensis* in the P2 treatment (pH 8.5 ± 0.2) were significantly higher than those in the P1 (pH 6.5 ± 0.2) and P3 treatments (pH 10.5 ± 0.2). According to Ismaiel (2016), the highest biomass of *Arthrospira platensis* is produced in media with a pH of 8.5-9.5. Although *Arthrospira platensis* can tolerate a wide pH range, a pH range farther from its optimal pH can reduce its growth rate. A low growth rate will also cause low biomass production.

Furthermore, the different salinity treatment factors showed that the maximum density and growth rate in treatment S3 (salinity of 20 ‰) were significantly higher compared to S1 (salinity of 0 ‰), S2 (salinity of 10 ‰) and S4 (salinity of 30 ‰) treatments. The S1 and S4 treatments were not significantly different and were the treatments that produced the lowest density compared to other treatments. Table 1 and Table 2 showed that the

highest density and daily growth rate was found in the S3 (salinity of 20 ‰) treatment. This is supported by the results of Kouhgardi et al. (2015), that *Arthrospira platensis* cultured on Conway media was able to produce the highest density of 912.07 mL<sup>-1</sup> cells at a salinity of 20 ‰. While the density and growth rate between S1 and S4 treatments showed no significant difference. This is because the salinity of 0-30 ‰ is still within the range of salinity that can be tolerated by *Arthrospira*. Ughy et al. (2015) said that *Arthrospira platensis* is one of the species of *Cyanobacteria* that can grow in an euryhaline environment.

	/p.		/		
Single Influence of pH (P)		Single Influen (LSD <sub>0,0</sub>	Main influence of pH $(P)$ 0.052		
P (- )	S1(0 ‰)	S2 (10 ‰)	S3 (20 ‰)	S4 (30 ‰)	- (LSD <sub>0,05</sub> =0.053)
P1 (pH 6.5)	0.433 a	0.633 <sup>b</sup>	0.767 <sup>cdef</sup>	0.467 <sup>a</sup>	0.575 <sup>a</sup>
P2 (pH 8.5)	$0.767 \ ^{cdef}$	0.833 <sup>ef</sup>	$0.867 \ ^{\mathrm{f}}$	$0.800  ^{\rm def}$	0.817 °
P3 (pH 10.5)	0.667 bc	0.733 bcde	$0.767 \ ^{cdef}$	$0.700 \ ^{bcd}$	0.717 <sup>b</sup>
Main influence of Salinity (S) (LSD <sub>0.05</sub> =0.062)	0.622 ª	0.733 <sup>b</sup>	0.800 °	0.656 <sup>a</sup>	
Table 2 The growth rate of	of Arthrospira	<i>platensis</i> cultur	ed in pH and sa	linity treatment	(% day <sup>-1</sup> )
Single Influence of nH (P)		Single Influen (LSD <sub>0,0</sub>	Main influence of pH $(P)$ 1 (12)		
pri (i )	S1(0 ‰)	S2 (10 ‰)	S3 (20 ‰)	S4 (30 ‰)	- (LSD <sub>0,05</sub> =1.612)
P1 (pH 6.5)	14.333 ª	18.659 <sup>cd</sup>	19.192 cde	13.348 ª	16.383 <sup>a</sup>
P2 (pH 8.5)	18.343 <sup>bcd</sup>	20.570 de	22.026 °	20.723 de	20.416 <sup>b</sup>
P3 (pH 10.5)	20.023 e	19.527 <sup>cde</sup>	20.623 de	16.417 abc	19.147 <sup>b</sup>
Main influence of Salinity (S) (LSD <sub>0,05</sub> =1.861)	17.566 ª	19.585 <sup>b</sup>	20.614 <sup>b</sup>	16.829 ª	

Table 1 Maximum density of Arthrospira platensis ( $g L^{-1}$ )

The interaction between pH and salinity factors showed that the density and growth rate in treatment P2S3 (pH 8.5  $\pm$  0.2 and salinity of 20 ‰) were significantly higher compared to other treatments. At maximum density, treatment P2S3 was not significantly different from treatments such as P1S3, P2S1, P2S2, P2S4, and P3S3. While in the growth rate, treatment P2S3 was not significantly different from P1S3, P2S2, P2S4, P3S2 and treatments P3S3. Table 1 and Table 2 show that treatment P2 (pH 8.5  $\pm$  0.2) is more dominant causing higher density and growth rate of *Arthrospira platensis* despite being in different salinity ranges. As for the treatments P1 (pH 6.5  $\pm$  0.2) and P3 (pH 10.5  $\pm$  0.2) provide the highest density when combined by the treatment S3 (salinity 20 ppt). The treatment of P1, P2, and P3 (pH 6.5  $\pm$  0.2, 8.5  $\pm$  0.2, and 10, 5  $\pm$  0.2) and S1, S2, S3 and S4 treatments (salinity of 0 ‰, 10 ‰, 20 ‰, and 30 ‰) still support the growth of *Arthrospira platensis* with the best treatment found in the combined treatment P2S3 (pH 8.5  $\pm$  0.2 with salinity of 20 ‰).

Mismatch of pH will cause lysis and can change the shape of pigment growth (Hariyati, 2008). The process of photosynthesis affects the pH value. In daylight, aquatic plants release carbon dioxide from water for use in photosynthesis. The release of carbon dioxide by plants occurs through respiration. When carbon dioxide is released, carbonate builds up and hydrolyzed so that the pH of the water will increase (Boyd, 1990). Prasadi (2018) showed that growth of Arthrospira could be inhibited if it was in the pH range above 10.5 or less than 7. Salinity is one of the factors that can influence osmotic pressure for *Arthrospira* as like as others of microalgae. Pisal and Lele (2005) microalgae can experience cell shrinkage in conditions outside the cell salinity higher than inside the cell (hypertonic), and vice versa under conditions of low salinity outside the cell (hypotonic) cell swelling will occur due to water molecules outside moves into the cell. This condition affects the process of photosynthesis, and makes microalgae to produce secondary metabolites in the form of β-carotene to sustain life against changes in salinity in culture media. While in isotonic conditions, cell fluid is isotonic to its external media which causes low active ion transport and osmoefector exchange, making the Na-K-ATPase enzyme activity at a maximum level and more energy will be utilized for growth (Rahmawati et al., 2012). The optimal combination of pH and salinity causes the growth of Arthrospira to be maximal. The optimal salinity range for Arthrospira is between 15-20 ‰, from the related research showed that the results of Arthrospira culture with 20 ‰ of media salinity, pH 7.5-8.5 using fertilizer media (0.010 g L<sup>-1</sup> TSP, 0.030 g L<sup>-1</sup> Urea, and 0.030 g L<sup>-1</sup> ZA) and a culture periode of 9 days produced a dry weight of Arthrospira of 0.0375 g L<sup>-1</sup> (Prasadi, 2018).

Rendement of phycocyanin

The rendement of *Arthrospira* was presented in Table 3. The pH condition of maintenance media can be affect of protein content in *Arthrospira* cells. The results of LSD <sub>0.05</sub> on the main factor of pH showed that the rendement of phycocyanin *Arthrospira* in treatment P2 was significantly higher compared to other treatments. Taufiqurrahmi et al. (2017), the amount of *Arthrospira* biomass influences the high content of phycocyanin. Table1 showed that the highest of *Arthrospira* biomass was found in treatment P2. It showed that the highest *Arthrospira* biomass produced the highest rendement of phycocyanin (Table 3). The culture medium of *Arthrospira* pH of 8.5 produced the highest C-phycocyanin content (Ismaiel et al., 2016). Rahmawati et al. (2017) said that the higher of C-phycocyanin followed the higher of rendement of phycocyanin.

LSD <sub>0.05</sub> showed that the main factor of salinity showed the rendement of phycocyanin in treatment S3 (salinity of 20 ‰) was significantly higher than other treatments. It is thought that the difference in salinity treatment has an impact on the external osmotic pressure of *Arthrospira* cells which results in changes in cell composition especially phycocyanin. Sodium will flow into the cell and cause the release of phycobilin (phycoeritrin, phycocyanin and allophycocyanin) from PS II (Photosystem II) and stop the electrons transporting to PS I (Photosystem I) followed by activation of the protective mechanism. *Arthrospira* will produce carbohydrates to balance intracellular osmotic pressure and require more energy to remove sodium ions from cells. In this case it will produce ammonium assimilation causes inhibition of protein synthesis (Zhou et al., 2017).

Single Influence of		Single Influe (LSD	Main influence of pH (P)		
p11 (1 )	S1	S2	S3	S4	(LSD 0,05=0.096)
P1	7.881 <sup>a</sup>	8.783 °	9.441 <sup>d</sup>	8.387 <sup>b</sup>	8.623 <sup>a</sup>
P2	9.657 °	10.906 <sup>h</sup>	11.347 <sup>i</sup>	10.423 <sup>g</sup>	10.583 °
P3	8.970 °	9.408 <sup>d</sup>	10.134 $^{\rm f}$	9.262 <sup>d</sup>	9.444 <sup>b</sup>
Main influence of Salinity (S) (LSD <sub>0,05</sub> =0.111)	8.836 ª	9.699 °	10.307 <sup>d</sup>	9.357 <sup>b</sup>	

Table 3 Phy	cocyanin (%)	yield in Ar	<i>hrospira</i> dry	biomass at 8 da	ys after inoculation
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The environment includes the availability of nutrients, pH, salinity, light and temperature can affect the growth and accumulation of biopigments from microalgae (Sharma and Tiwari, 2014). The condition of culture media is able to influence the growth phase of *Arthrospira*, causing changes in the composition and proportion of phycobilin (phycoeritrin, phycocyanin and allophycocyanin) (Simeunovic et al., 2013). The results of the LSD 0.05 was showed that the rendement of phycocyanin *Arthrospira* on the interaction between factors in treatment P2S3 was significantly higher than in other treatments, but it was not significantly different from treatment P2S2. This showed from the density and growth rate of *Arthrospira*. The increasing salinity will cause maintenance media to be hypertonic towards cells and result in poor absorption of nutrients by cells. These cells could reduce in protein and increase in carbohydrates from *Arthrospira* cells (Ravelonandro et al., 2011).

Production of phycocyanin was able to reach 12.4 % - 17.6% of biomass dry weight of Arthrospira cultured in Zarrouk Medium (ZM) (Prates et al., 2018; Garcia-Lopez et al., 2020). There are several factors that affect the rendement of phycocyanin include temperature, extraction time, mixing rate, biomass, type of solvent and the ratio of biomass to the solvent (Taufiqurrahmi et al., 2016). The content of phycocyanin in cyanobacteria increases when grown in low light intensity. Phycocyanin is a pigment associated with protein, polar and water soluble. The protein content of microalgae are influenced by environmental conditions such as temperature, age of culture, light intensity, pH, salinity, and nutrient limits (especially nitrogen). Nitrogen is an essential element needed for the synthesis of accessory pigments and chlorophyll. When microalgae are growing fast, it requires large amounts of nitrogen and could consume phycocyanin as an alternative source of nitrogen for the production of biomass (Hsieh-Lo et al., 2019). It must be optimized for biomass production and phycocianin content. The higher concentration of phycocyanin will be followed by the rendement of phycocyanin. In this study, the phycocyanin content is lower than previous study, because of different media for culturing Arthrospira. The nutrient from ZM (pro analysis substances) is more complete for growing and forming phycocyanin than waste water catfish pond media, especially the trace mineral in ZM.

### **Reduction of Total Nitrogen and Phosphate Content**

The percentage reduction of total nitrogen in the culture media was presented in Table 4.

Single Influence of		Main influence of pH (P)			
pri (i )	S1	S2	S3	S4	(LSD 0,05=0.645)
P1	80.990 <sup>a</sup>	82.250 ab	83.767 cde	81.897 <sup>ab</sup>	82.226 ª
P2	83.880 de	84.377 ef	85.420 f	84.857 <sup>ef</sup>	84.633 °
P3	82.940 <sup>bcd</sup>	81.143 <sup>a</sup>	84.950 ef	84.813 <sup>ef</sup>	83.462 <sup>b</sup>
Main influence of Salinity (S) (LSD <sub>0,05</sub> =0.745)	82.590 ª	83.856 <sup>b</sup>	84.712 °	82.603 <sup>a</sup>	

Table 4 Reduction of total nitrogen content in Arthrospira culture medium (%)

The mechanism for removing nitrogen in water is determined by several factors, including bacterial activity (Gersberg et al., 1986), uptake by plants (Breen, 1990) and evaporation (Sanchez-Monedero et al., 2001). In Cyanobacteria, nitrogen is a macronutrient that plays an important role in the formation of biochemical compounds such as nucleic acids (DNA, RNA), amino acids (protein) and pigments (chlorophyll and phycocyanin) (Markou et al., 2014). The results of the LSD <sub>0.05</sub> on the main pH factor showed that the reduction in total nitrogen content in *Arthrospira* culture media in treatment P2 was significantly higher than in treatments P1 and P3. This is due to the higher density of *Arthrospira* in treatment P2 compared to other treatments, so that the utilization of nitrogen by *Arthrospira* in culture media is greater than others. Markou et al. (2014) showed that the higher density of *Arthrospira* could be higher the absorption of nutrients including nitrogen.

The value of reducing total nitrogen content in S3 treatment was significantly higher than for other treatments. This showed that treatment S3 caused *Arthrospira* to absorb nitrogen higher than other treatments in line with the high density and rendement of phycocyanin *Arthrospira* obtained in this study. Jabeen and Ahmad (2011) showed that salinity in culture media influences nitrogen absorption and protein biosynthesis. Reduction of total nitrogen content in *Arthrospira* culture media due to interactions between pH and salinity factors showed that treatment P2S3 was significantly higher than other treatments. With the optimal conditions (pH and salinity), *Arthrospira* is able to make maximum use of nitrogen. This can be seen from the highest density, growth rate and rendement of phycocyanin found in treatment P2S3. The amount of nitrate and phosphate decreases with increasing growth of *Chlorella vulgaris* and *Arthrospira platensis* (Sayadi et al., 2016). This is because algae have the ability to absorb nutrients such as nitrogen and phosphate are use to carry out photosynthesis and protein production. Reduction of phosphorus content in waters is influenced by the process of absorption, complexation, deposition and assimilation (between microbes and plant biomass) (Tanner et al., 1999).

Single Influence of		Single Influen	Main influence of pH		
pri (r )	S1	S2	S3	S4	_ (I)
P1	70.500	71.667	73.000	72.333	71.875
P2	74.667	74.000	74.667	72.667	74.000
P3	70.333	70.333	73.667	72.667	71.750
Main influence of Salinity (S)	71.833	72.000	73.778	72.556	

 Table 5
 Reduction of phosphate content in Arthrospira culture medium (%)

The results of LSD  $_{0.05}$  on the main factor of pH showed that each treatment did not significantly affect the reduction of phosphate content in *Arthrospira* culture medium. Plants can only absorb phosphorus in the form of H<sub>2</sub>PO<sub>4</sub> and HPO<sub>4</sub><sup>-2</sup> free orthophosphate ions (Becquer et al., 2014). The orthophosphate content decreases with increasing media pH. Cerozi and Fitzsimmons (2016) showed the orthophosphate content increases in the pH range from 5.5 to 8.5 and decreases when pH 10. The fall in the orthophosphate content at pH 10 is due to an increase in calcium phosphate formation. The value of reducing the phosphate content in *Arthrospira* culture medium was presented in Table 5. The salinity factor, administration of different salinity in each treatment has no significant effect on reducing the phosphate content. Bassin et al. (2011) explained the reduction of phosphorus will be inhibited when a combination of Cl<sup>-</sup> and nitrite and Cl<sup>-</sup>concentration more than 2.5 g L<sup>-1</sup>. The interaction effect of differences in pH and salinity, each treatment had no significant effect on reducing phosphate content. Hua-Sheng et al. (1995) showed that the utilization of Dissolved Organic Phosphorus (DOP) can be through active uptake into cells or by extracellular mineralization by phosphatase enzymes. However, most DOP compounds cannot be assimilated directly with microalgae because they have been mineralized. Markou *et al.* (2014) stated that phosphorus is a macronutrient that plays an important role in the preparation of nucleic acids (RNA and DNA), phospholipids and energy-carrying molecules (ATP). The phosphorus content in plants is lower than Ca, N, and K (Sasaqi et al., 2018). Although the analysis of variance shows that the results are not significantly different, but the highest phosphate reduction still existed in treatment P2S3.

## Morphology of Arthrospira in various pH and salinity media

Morphology of *Arthrospira* was affected by increasing or decreasing physical or chemical factors in their culture media. Salinity and acidity value combination at this study didn't change the filament of *Arthrospira* significantly as their filament of *Arthrospira* under microscope with 100x magnification (Figure 7). The morphological form of Arthrospira were not different in all of pH and salinity treatments. The waste water catfish pond media could affected in the linierizing filament. This study indicated that salinity and acidity of culture media didn't effect on various form filament, either straight and helical.

The straight filaments were observed for *Arthrospira* strains during two years of cultivation, and their presence in *Arthrospira* sp. Nigrita C1 cultures was constant. The various morphological plasticity, greatly influenced by the growth stage and exogenous parameters, such as temperature and light intensity, was observed for *Arthrospira* strains (Papapanagiotou and Gkelis, 2019). There are indications that adaptability to change in environmental conditions is relatively rapid and also subsequent changes at the genetic level can be realized quickly. This means that we can easily find different genotypes in various stable, ecologically different habitats. Morphologically similar strains were cultured for a long time under uniform and stable conditions (Komarek, 2016). But the morphological changes couldnot be effected by acidity and salinity of culture media.



P1S4



P2S4

P3S4

Figure 7. Morphology of *Arthrospira* in catfish wastewater culture media at several treatment of pH and salinity

## Conclusions

Arthrospira that is cultured on waste media (observed in liquid culture) indicated some short and linear filaments. Identified Arthrospira had a genetic distance of 6.8% between AF and AW isolates. AF isolates had a close relationship with *Arthrospira platensis* petH species originating from Japan (bootstrap value of 95%) while AW isolates form phylogenetic branches which are separated from AF isolates and *Arthrospira platensis* petH species originating from Japan (bootstrap value 85%). The catfish culture wastewater media at different pH and salinity affects the density, growth rate and rendement of phycocyanin *Arthrospira platensis*. The highest density, growth rate and rendement of 22.026% day<sup>-1</sup> and the rendement of phycocyanin of 11.334 %.

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# SUBMISSION CHECKLIST

Ensure that the following items are present:

The first corresponding author must be accompanied with contact details:	Give mark (X)
• E-mail address	Х
• Full postal address (incl street name and number (location), city, postal code, state/province, country)	х
Phone and facsimile numbers (incl country phone code)	Х

All necessary files have been uploaded, and contain:

٠	Keywords	Х
•	Running titles	Х
•	All figure captions	Х
•	All tables (incl title and note/description)	X

Further considerations

• Manuscript has been "spell & grammar-checked" Better, if it is re	vised by a professional
science editor or a native English speaker	
• References are in the correct format for this journal	Х
• All references mentioned in the Reference list are cited in the text, a	nd vice versa x
• Colored figures are only used if the information in the text may	be losing without those x
images	
• Charts (graphs and diagrams) are drawn in black and white i	mages; use shading to x
differentiate	

## Bukti konfirmasi review dan hasil review pertama (21 November 2020)



Marini Wijayanti <mariniwijayanti@fp.unsrl.ac.id>

## [biodiv] Editor Decision

Smujo Editors <smujo.id@gmail.com>

21 November 2020 pukul 08.39

Balas Ke: Smujo Editors <editors@smujo.id> Kepada: Marini - Wijayanti <mariniwijayanti@tp.unsri.ac.id>, Mochamad Syafludin <msyalfudin76@gmail.com>, Yulisman Yulisman <yul\_cancer@yahoo.com>, Nuni Gofar <gofamuni@gmail.com>, Yuliy Nurianti <nuriantiyully88@gmail.com>, Anita Hidayani <antitahidayani30@gmail.com>

Marini - Wijayanti, Mochamad Syaifudin, Yulisman Yulisman, Nuni Gofar, Yuliy Nurianti, Anita Hidayani:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "The Characterization of Arthrospira cultured in waste water of Clarias catfish farming mediaanin: DNA barcode, helical form, growth, and phycocyanin".

Our decision is: Revisions Required

Smujo Editors editors@smujo.id

### Reviewer C:

The aim of this study was to characterize the morphological forms and DNA barcode based on 16 rRNA gene of Spirulina cultured in fertilizer and waste water effluent of Clarias pond farming media and determining optimal pH value and salinity of culture media for growth and C-PC production. The authors carried out so many experiments and analysis and extracted so many data which are appreciated. They worked on morphology of Arthrospira cultured with fertilizer and waste water of the fish. they compared the results in some of the characters with each other but in some other characters no.

there are two main problems in this experiment which are:

1- There is no control+)- to compare the results of growth, phycocyanin and identity blasts. The authors need to bring the results of CA (commercial Arthorospira) and AF to see the differences between the treatments with Control. the comparison only in morphology has been brought. They should bring this comparison in the other factors they worked like growth, phycocyanin, N and P reduction etc.

2- Still it's not clear how the pH and salinity along with some other factors in the waste water can change genome of an organism during a week.

Recommendation: Revisions Required

Reviewer V:

- What the source of Anthrospira?
- Why not use a specific Zarrouk medium?
- In Table 1 and 2

Salinity 10,20 and 30% means 100,200 and 30 grams per liter is very high and this under stress which means the reduction of growth rate.

- How is this given growth rate?
- What are the components of wastewater?
   Figures 4 is not clear, should be redrawn for high quality.
- Pigures 4 is not clear, should be redrawn for right quality.
   7- Attention to references

8 - There are some notes in the text

Recommendation: See Comments

Biodiversitas Journal of Biological Diversity

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Characterization of Arthrospira platensis cultured in waste water of Clarias catfish farming media: DNA barcode, helical form, growth, and phycocyanin

Abstract, dirthrospiral production sechnology in entfish seate media can be an alternative to reduce environmental pollution. However, some environmental factors such as mutrinon, light and water control can influence characterizations of dreforaptive at the genetic and physiologic level. dreforaptive photomics is one of the physicocyanin-producing crossedurateria and can be cultured using entfish estimation waterweater. Water quadrative speciality phil and salinity can effect of growth rate and undement of physicocyanin from dreforaptive photomics is one of the physicocyanin-producing crossedurateria in this study and the Gonsthank based on the 165 (RNA gene, and determine the optication of phil med salinity regards to be the psodimic of cattifue colume waterweater is physicocyanin maximum production of *dreforaptive*. The optimization of phil and salinity method used Completely Randomized Design (CRD) factorial with 2 factors consisting of the first factor with 3 reatments and the second factor with 4 treatments and 3 replications. The first factor wave salinity (see salinity of the first factor with 3 or physicocyanin, and decreased of total mitrogen and 3 physications. The first factor wave salinity of the first factor wave physication of *dreforaptive* and the second factor with 4 treatments and 3 replications. The first factor wave phil of callure median is due, phil physication (see 5), 000, 103, 20 ppr and 30 ppr, Parameters observation in colume median, that were salinity 0 ppr). Read-pt physication (see 5), 000, (8 45%), https://glugencit.trees.indicated that A had a longer and holds. Block failoware compared to *dreforaptive callured* on swater media (AW) which showed sveral linear and should be see 163, isolates and *dreforaptive philomytic* petit firm. Japan theoremizery value of 5%). We be true mindicated that A had a longer read-block failoware compared to *dreforaptive callured* 0.0.8673 grams maximum density, growth rate of 22.020 %, day 4 isolates and *dreforaptive philomytic* petit firm. Jap

Reprinted satisfic culture waterwater, DNA harvoile, pH, physicspanin, phylogenetic analysis, salinity, Asirolou( tritter and sol VAL DR.R.

### INTRODUCTION

Arthrospins is a genus of cyanobacterial microalgar, commonly known under the incomonically incorrect brand more Spiralina' (Papaparagiotou & Calelia, 2019). The cyanobacterial genus drifteringing Silveinberger as Geniool 18% contains at pressure 33 apecies, along with 12 infraspecific taxa (Guiry & Caler, 2018). and 2021 infrastrumon 1 My contains at pressure 33 apecies, along with 12 infraspecific taxa (Guiry & Caler, 2018). The synchrotread on polyphasic approach. Varians genetypes are adaptable to various approach, so which must be added other criteria tromphological, acological) if they are available and which are dissingt and recognizable in cyanobacterial populations (Komarch, 2016). A polyphasic approach, Varians genetypes are adaptable to various approach, so which must be added other criteria tromphological, acological) if they are available and which are dissingt and recognizable in cyanobacterial populations (Komarch, 2018). A polyphasic approach, Varians genetypes and adaptable to an expression of the added other criteria tromphological, acological) if they are available and which are dissingt and recognizable in cyanobacterial populations (Komarch, 2018). Received and the synthesis approach with a data of the synthesis (Komarch, 2018). Recent studies have above that Arthrospin on the need for treating waterwater, and approach of the other (Zhong et al., 2009 ar 2019). Industrial and processing waters and by-president for culturing *Scientifus* (*Activerophice*) at 2018, widyamisers et al. 2019). Aquisculture could apply an integrated strategy of simultaneously training analytical strategy of simulation to an advectory of 2018 are advectorial et al. 2019). Aquisculture could apply an integrated strategy of simultaneously training apparallel and the producing the biomass is a supplement field detter. The intrinset composition in their biomass degends on their erfluent which producing the biomass. Their character could be different with the various media for growth.

Basically, Arthrospiru's morphology is characterized by trichomes that circular regularly (helical). However, absorbal merphology can also necur in Arthrospiru as a circular shape that is irregular even linear. In some cultivation conditions, linear filaments can apontaneously terum to the belis, However, there are significant differences in morphology, ultrastructure, physiology, biochemistry, and genetic characteristics between the original filament and the latear filament but not the difference between the original and the returned filament. Linearization in Arthrospiru is a variation on the genetic level that can be caused by several environmental factors such as natrition, light and content of water modils for growth (Wang and Zhuo 2005). Accending to Liu et al., (2016), DNA barcoding has developed as a reliable tuchnology for identifying species based on variations in the sequence of standard DNA regime. This method is used successfully in a variety of biological applications including finding cryptic species, detecting invaries species, and identifying plants. DNA barcoding is a simple sheet genome sequence amplified via PCR using appropriate priori. Advances regimes to the original in the 165 rRNA gene has been widely used to determine batterial DNA characterization. Therefore, idemtification of Arthrospora using the 168 rRNA gene needs to be done to got the characterization of Arthrospora that is cultured on technical femilizer and waste media and determine the phylogenetic tree structure that has been recorded in Gentlank. Genflunk.

Culture of Arthroughra (Spirialise) in Claritar poind farming wasted water could have specific characterization to optimul pH value and salinity. Their adaptation to grow in organic water water make charge in bioactive and important compound production. Their biomass has a mutricolar state of \$5,70% procession, 6-10% ligid, 20% carbobydrate, besides being rich in minerals, vitamins, and pigments (Borowitzka et al., 2016; Vernes et al., 2016; ar 2015). Some color pigments that can be produced such as physicocyanin (blue pigment), alkephysicocyanin thear-green and physoerythrin (red pigment) (Sharma and Tiwori, 2011; Arrene et al., 2016; a source of food codering, custoreline, following which has functions as an antiovisidant (Pirnamyo and Limanture, 2008); a source of food codering, custoreline (Line et al., 2012). Coll of the factors that infhaence physoecyanin levels is biomass (Taufigurethmi et al., 2017). The pH and alimity of cubine modific cubic that infhaence physoecyanin (Clarity 1983) Marce et al., 1987; Planes et al., 2002). Israici et al. (2016) the biomass that infhaence physoecyanin (Clarity 1983) influence et al., 1987; Planes et al., 2002). Israici et al. (2016) the biomass the biomass of Arthropytor (Clarity, 1983) Marce et al., 1987; Planes et al., 2002). Israici et al. (2016) the optimal et al. (2016) the optimal pH value for growth of ervitonmental factors, especially salinity, influence the productivity of cell biomass, photosynthesia, shape, and flow of echalar metabolic activity that affect the dynamics of cell composition (Hu, 2004). The optimal pH value for growth or elabalism det distruming used in the dynamics of cell composition (Hu, 2004). The point physical and fit value of *Arthropowe* cubiner media have been known to affect the morphology of the filament. The aims of this study is characterizing morphological forms and DNA baside based on the 185 rfNA gase of *Arthropowe* (Spirulina) cubinered in fertilizer and wasee water refluent of Clarices pond farming water me Culture of Artheosphy (Spiruline) is Clariar pond farming wasted water could have specific characterization for

### MATERIALS AND METHODS

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EXAC accountant DNA extraction was carried out according to procedures in which there was a Presto TM Mini gDNA Bacteria Kit (Genenia Biotech E.d.). DNA extraction consisted of several stages: sartple preparation, lysis, parifloation, and precipitation or webing. The sample used was 0.15 grams of wet weight for one extraction <u>(Whate is the reformer?)</u> 106 107 108

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DNA amplification The process of DNA amplification using the PCR (Polymerase Chain Reaction) method was performed using 2 μl forward primers 031(5)-CAGICCC TAA CAC ATU CAA GTC-3.1 and reverse primer 1387; (3)-GOU COO WOT GTA CAA GGC-3.2 (Marubesi et al., 1999). The total compression of the PCR mixture was 50 μl which consisted of 25 μl Go Taa Green, 12 μl NFW (Nuclease Free Water) and 8 μl Arthrophysic Acturation template. DNA amplification was carried out in stagars; the initiation cycle at 95% for 5 minutes, followed by 36 denotarization cycles at 94 %C for 30 seconds, amending at 55 % for 30 seconds, then the extension stage at 72 %C for 1 minute, and the final stage 72 %C for 7 minutes (Lee et al., 2003). 109 110 111 112 113 114 115 116

Electrophoresis was carried out using 1% agarose gel at 75 V for 35 minutes. Agarose that have been electrophoretis was immersed with a mixture of 10 µl diamond dye solution and 100 ml TAE 1x huffer solution for 30 minutes without exposure to light. The results were visualized through gel documentation by observing DNA migration using a transitiumizate UV. 117 118 119 120 121

Gene Sequences UV.
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Gene Sequences III.
Arthrospica DNA samples that were successfully amplified using PCR were then sequenced in the fragments of 195-rfbNA gene. The amplified products were sequenced through the services of the Macrogen landstate. The DNA sequences obtained in the form of feats format were aligned using MEGA 6.0 sufbare and the long obtained the form of feats format were aligned using MEGA 6.0 sufbare and the uploaded through the land to Local Alignment Search Tool (BLAST) program, BLAST was a program to search for and analyze the homology of an organize's sequences obtained in the form of feats format gene where is in that its homology can be sekretified with other genus Arthrospira 105 rfbNA gene, constructed using the Neighbor-Joining (NJ) method. The phylogenetic tree was constructed through the form of images and discussed descriptively by referring to the appropriate literature. Continuous of pH and adinny for optimizing pH and adinity metha for growing Arthrospira in Caffah farming wasted water interview approximal of reprinting pH and adinity metha for growing Arthrospira in Caffah farming wasted water interview of the first for optimizing pH and adinity metha for growing Arthrospira in Caffah farming wasted water into a caffah farma (16 form of pH in culture and high phylogenetize). Communication of pH and adinity for epitomized Design (CRD) consisting of the first farming wasted water was a Fartorial Complexity PL culture media pH is 0.5 ± 0.2. The Section IC Complexity of 10 %s, S3: salinity of 10 %s, S3: salinity of 20 %s, and S4: salinity of 30 %s (What is Metrone of salid).

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Continue programming Continue programming and in this study was sterifized using 70% a doubled to minimize the contaminants that ishibit the productively of Arthropolar, The comminants issued plastic buttles with capacity of 5.1, volume of 36 nucles. The plastic bottle productively of Arthropolar, The comminants issued plastic buttles with capacity of 5.1, volume of 36 nucles. The plastic bottle issues sterificed using a polarisation permangament solution (2, ng, 1, <sup>3</sup>). Califab cellure wertexiater obtained from estifish farming portion structure with 150 grants fishe<sup>3</sup>, maintained for 2 seconds by providing artificial food (protein 31%-53%), while the strend was structure with solutions to treatments 51, 52, 53 and 54 were added with all one doed, while the strend was between two is treated with solitions. In treatments 51, 52, 55 and 54 were added with all one of 11C1 1 N of 0.75 mL<sup>3</sup>, <sup>11</sup> in P1 treatment to make apt of 0.5. Meanwhile, in treatments P2 and P3, are get a pH of 8.5 and pH 10.5 there was an addition of NaOH 8 N as much as 0.07 ml L<sup>3</sup> and 0.45 ml L<sup>4</sup>.





### 167

Arthrospira cultivation. Arthrospira previously used was cubured in catfish culture wastewater for cubure stock with a density of 2 g L<sup>3</sup>. The stock was taken as much as 400 ml in 3600 ml of catfish culture wastewater in accordance with treatment. Attainin was used for agitation, the lighting using 36 wart TL lamps for 24 hours day<sup>-1</sup> during maintenance (Figure 2). Harvest of biomass, <u>What an the components of wastewater</u> Harvest of the biomass was after exponential phase by filtering. The biomass was dried using an oven for 14 hours at 40 % (Afrian et al., 2018 not found in the reference, with modification). The dry biomass was used for the phycocyanin extraction process. 168 169 170

- 172 173 174

### 175 Phycocyanin extraction 176

171

The dry biomass was 0.04 g added by 1 ml of phosphate buffer pH 7, then homogenized and frozen in the freezer for 24 hours at a temperature of -4 °C. After 24 hours from the freezer, thawing process for 15 minutes. Samples were centrifuged for 30 minutes at 3000 rpm. After that, the sodiment and the superratant ware separated. The resulting supermatant was phycocyanin which he analyzed using the Bennett and Bogorad method (1973). 178 179 180

## 151

The density of Arikonopira humans. Biomass density measurements were performed at each treatment and 3 replications every day at the same hour. The density of humans was 1 ml of sample in each treatment with 3 replications. The 1 ml of sample into aluminum bowl. The sample and the aluminum bowl were weighed, then driad in the oven for 14 hours at 40 °C. The sample of water that had 182 183 184 185 186

dried was weighed again. The dry biomass weight of Arthrospira biomass was converted to g L



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189	Figure	A Dty biomen of Arthropping glatents after oven
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191	The	growth rate of Arthrosphru can be calculated using the following formula according to Vonshak (1997):
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193		$\mu = \ln N_t - \ln N_0 \times 100\%$
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196	Note	μ = daily growth rate (7% days")
197		t = time (daya) from Na to N.
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204	1.04	measurement of processing refers to deniate and respond (1973). The amornou supervaluation was measured using
200	a spece	opsolutierer at wavelengtits of 015 min and 052 min.
2007		C absorbance and the011.6151, 0.474 (010.657)
208		5.14
2000		Read-second distances in the second sec
710		Residentiation of physics yanta (reg g 7) - Lettering yanta (reg g 7)
211		Rendoment percentage of phycocyania (%) = Rendement of phycocyania (ing g *) a 100%
212	Note :	C-phycocyanin = C-phycocyanin contamination (ing, mL-1)
213		V = Solvent Volume (ml)
214		DB1 – D(y Biomass (0.04 g)
215		0.474 and 2.34 <sup>or</sup> coefficient of extanction (Benneti and Bogurad, 1973)
210	11.1	works were advantantly a structure of endering the second statement of the second s
217	100	results were successed to sumple analysis of variance tests (ANOVA) (p 90.05) and in the case of significant
218	different of the second	tees, the means were compared by the Least Segniticant Differences fast (p+0.05).

differences, the means were compared by the Least Significant Differences test (p=0.05).

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The results of the identification of isolates showed that the isolate had a twisted filament shape resembling a spiral (helical). Based on Davity identification book (1955), it is known that the isolate used in the unaly was *Spiralias (Arthrospira) platestis. Arthrospira*: is cyanobacteria belonging to the order Oscillatoriales which has a filament (trichome) that resembles a spiral (helical) but does not have beterocyst cells (Sze, 1998). Heterocyst cells are special thick-walled cells that play a role in nitrogen fixation from the air (forger et al., 1979, nut found in the reference). In this shape resembles a spiral (helical) but does not have beterocyst cells (Sze, 1998). Heterocyst cells are special thick-walled cells that play a role in nitrogen fixation from the air (forger et al., 1979, nut found in the reference). In this study *Arthrospira* caltured in different media had several licear/straight morphologies. Based on Figure 2, *Arthrospira* which was cultured on technical fertilizer media has a longer and spiraling morphological form compared to another cultured on waste media. Their filaments have more linear morphological form, some spirals but not too long. According to Astiani et al (2010), *Arthrospira* growth is influenced by natrition and environmental factors. Wang and Zhao (2005) explained that linearization that occurs in *Arthrospira* is a variation of the genetic level that can be cameed by environmental factors such as lack of mitrition and high light intensity. In this study, isolates were cultured with the same filaments of 0.24 hours. Linear filaments in AW have a lower metabolic rate compared to befical filaments. This is one of the adaptive mechanisms for *Arthrospira* to survive some environmental conditions that are not appropriate.

Tables. 1. The results of the BLASTn analysis of derivergeine annuples cultivated in technical firtilizer and wante mediume with data

Description	Identity (%)	Access ends	Sample arigin
Arthrospina (fortilizer)		1.1852.000	South a market
Arthrospira platensis patH	100	AB113346	Jagan
Spirulina platensis CCC 478	90.48	JX014313.1	lindia
Spiraling platennic coold	94.4	D49531.1	James
Arthronomica phatennia PCC 7345	90,12	JN831264.1	USA
Arthropping maxima EEW2	74.4	190008225	Australia
Arthronpica (wante modia)			
Arthropping plateouir petiti	94.3	D49531.1	Jagmen
Arthropping platensis DKCAS2	81.4	MG912588.1	Tradict
Spiruling platensis CCC 478	74.4	J20014313.1	Institut
Inthrosping waxing str. Lefevre 1963/M-132-1	73.3	FJ798612	Vonemaoilla
Arthrospira maxima EEW2	72.2	HQ008225	Austicelia

Phylogenetic Tree The results of the 165 rRNA encoding gene sequences from AF and AW isolates were traced to other Arthrospiral isolates present in GeneBlank through the BLAST program. The results of the BLASTs analysis of Arthrospiral samples cultivated in technical fertilizer and waste media with dats in Genbank are postented in Table 1. Table 1 results of the BLAST analysis show the closeness between AF and AW isolates with other isolates in GenBank. It show that Arthrospiru technical fertilizer isolates and Arthrospiru waste isolates have the closeness homiology to Arthrospiru plateous peth species from Japan with percentage values respectively 100% and 94,3%.



Figure 5. Phylogenetic analysis with 1000 biomings AW (*Arthrogenes* subtrend in waste water media) and AP (*Arthrogenes* subtrend in facilities media)

Fertiliser anothil
Genetic distance was used to see kinship relationships from Arthrospice both AF and AW samples with sequential data from Genbank. AF isolate indicated a genetic distance of 0.068 with AW isolates. AW and AF isolates showed the lowest genetic distance respectively 0.089 and 0.000 with Arthrospice platentis pettl from Japan. Analysis bused on genetic distance showed that both isolates were heleng to the name species namely *Southwas*, however the genetic distance respectively 0.089. The phylogenetic true Arthrospicra platentis pettl from support and 0.080 (KM) meaning that there are immerspecies variations in the sample canad by mutation.
The phylogenetic true is a two-dimensional graph showing relationships between organisms or population classifications based on the vertex or phylogenetic true is a two-dimensional graph showing relationships between organisms or population classifications based on their evolutionary biatoey. The result of phylogenetic true scentraction showed that both samples formed hranches with south explorements true from AF and AW isolate sequences formed cluster was separate with several other *Arthrospira* platoexis.
The Ardiani (2003) states that bootstrap analysis is performed to determine the level of confidence in grouping. Bootstrap value is formad to a cluster relationships between orthosis pettl species. Genetically, they had diverse, and salate a negative brack of AP isolates and diverse platowists pettle species. Genetically, they had diverse, and adapted to a separate track of AP isolate made to a separate brack of AP isolates and diverse platowists pettle species. Genetically, they had diverse, and adapted to a separate track of AP isolates and (2006) stated that species. Constituently collitions. The AW isolate indicated different coefficients can form a separate subcluster with a bootstrap value of OPs. The isolate has been as explored and AP isolate and different coefficients can form AP isolate and the species. Constraically, they

Density and Growth Rate of Arthroopirs cultured in nutressater of caffish farming The biomuss of Arthroopirm displayed mechanism of adaptation in culture media. The wastewater media could make different charateristic of growth as like as filament. The maximum densities of Arthrospire cultured in wasted water were achieved on a different day. The daily density of Arthrospirs during culture can be seen in Figure 6.



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Figure 6. Celli donsity (dry weight with a mointure connect of 1.2%) of "detheo sing in particle of

Figure 6. Cell density (day weight with a monute connect of 12%) of .trikropping in entities rearing pend water water.
The graph presented in Figure 6, it show that in the culture period from day 1 to day 2, Arthropping in each treatment of P252, P253, and P253 hast from day 1 to day 8 of the culture period. In this furnities of P252, P253, and P253 hast from day 1 to day 8 of the culture period. In this furnities of P252, P253, and P253 hast from day 1 to day 8 of the culture period. In the streatment of P251, P254, P351 and P254 bast of the culture period. The decreasing Arthropping density for the treatment of P251, P254, P351 bast day 0 the culture period. The decreasing Arthropping density for the day 1, P36, P351 bast of day 1, while the treatment of P251, P254, P351 bast day 1 to day 1, and experimences a stationary phase from day 7 to day 9 then extern the day 1 to the 91. The decreasing Arthropping vosible because of reducing the matients in the culture metal.
The maximum density of Arthropping could be achieved on different day, between 5 – 8 days after culturing. The maximum density of Arthropping could be achieved on different day, between 5 – 8 days after culturing. The maximum density of Arthropping could be achieved on different day, between 5 – 8 days after culturing. The maximum density of Arthropping could be achieved on different day, between 5 – 8 days after culturing are the achieved on differences in p14, within y between 5 – 8 days after culturing and the achieved for the probability of 0.00 g L - 4 day 1 (Nobey 1, 4 day 2, 10). The catfish rearing water water. The maximum density of Arthropping and the state of the probability of the 1.5 day at the state of the probability of 1.00 days 1, 4 day 1 (10.2 L - 10.2 M -

326 327 328 328 329 330

337 338 339 340 341

344 548 349

Table 1. Maximum density of dethrousing platomic (g.l.2)

Single Influence of pH (P)	Single Influence of Salinity (S) (LSD <sub>A.tt</sub> = 0, 107)				Main influence of pH (P)
	S1(0 %a)	32 (10 %c)	83 (20 %)	34 (30 %)	(LSD ear-0.053)
P1 (pH 6.5) P2 (pH 8.5) P3 (pH 10.5)	0.433 * 0.767 == 0.667 ==	0.833 # 0.833 # 0.733 ***	0.767 tale 0.867 f 0.767 tel	0.467 × 0.800 ml 0.700 md	0.575 × 0.817 × 0.717 ×
Main influence of Salinity (5) (ESD are=0.052)	0.622 *	0.733 *	6.800 *	0.656 *	

	S1(8 %)	52 (10 %)	4(1)(10(2)-)	Act (10, 201)	(1.50) a.m <sup>-1</sup> .012)
17 2 21				204 1249 2001	
41 8.5) 41 8.5) 41 10.5)	14,333 * 18,343 *** 20.023 **	18.659 H 20.570 ± 19.527=	22.026* 20.623 *	13.348 * 30.723 # 10.617 #	16.383 + 20.416 * 19.147 *
influence of Salinity	17.566*	19,585 *	20.614*	16.829.4	
	ALK 5) AL 10.5) indumee of Salinity (SD carri, 861) n Table 1 and 2 aligned 10 70 and 100	41.6.5) 15,143760 41.10.5) 20.023 44 influence of Salinity 17.566 - <u>SD asset1,8611</u> n Table 1 and 2 dimension 20 and 2005 protection 100.5	48.8.5) 15,143 <sup>144</sup> 20,570 ± 10,51 10,51 19,527 ± 10,507 ± 19,527 ± 10,507 ± 19,585 ± 10,507 ± 19,585 ± 10,507 ± 19,585 ± 10,100 ± 10,052 10,100 ± 10,1000 10,100 ± 10,1	48.85) 18,143% 20,570 4 22,026 4 810,53) 19,327% 19,327% 20,623 4 influence of Satisfield 17,566 9 19,585 4 20,614 4 <u>0,7146 1,566 2</u> <u>0,716 1,561 1</u> <u>0,716 1,565 1</u> <u>0,716 1</u> <u>0</u>	48.85) 18,143% 20.570 22,025 20.722 20.723 10,723 10,723 10,723 10,723 10,723 10,723 10,723 10,723 10,723 10,723 10,723 10,723 10,725 1

The interaction between pH and salinity factors showed that the density and growth rate in treatment P253 (pH 8.5 ± 0.5 mm of a gain/icantly different from F053, P251, P252, P254, P352 and F353, Toble 1 and Table 2 show that treatment P253 was not significantly different from P183, P252, P254, P352 and F353, Table 1 and Table 2 show that treatment P253 was not significantly images. As for the treatments P1 (P1 6.5 ± 0.2) and P35, P251, P254, P352 and treatments P383, Table 1 and Table 2 show that treatment P253 was not significantly different from P183, P252, P254, P352 and treatments P383, Table 1 and Table 2 show that treatment P253 was not significantly images. As for the treatments P1 (P1 6.5 ± 0.2) and P3 (pH 6.5 ± 0.2, and P3 (pH 6.5 ± 0.2) which are proved to the threatment (Salinity of 0 %, 10 %, 20 %, and P3 (pH 6.5 ± 0.2) which are proved to the threatment for the treatment of P1, P2, and P3 (pH 6.5 ± 0.2, 8.5 ± 0.2, and P3 (pH 6.5 ± 0.2) which are proved to the table of pigment growth (Hariyati, 2008). The process of photosynthesis affects the pH value. In charge the shape of pigment growth (Bariyati, 2008). The process of photosynthesis. The release of arrbon dioxide by plants occurs through respiration. When carbon dioxide is released, arbona builds up and hydrolyzyny are list as others of microalgae. Pisal and Leie (2005) microalgae can and process with of Arthronyour could be inhibited if it was in the pH range above 10.5 or less than 7. Salinity is one of the factors that can influence osenotic pressum for Arthropyirr an list as a others of microalgae. Pisal and Leie (2005) microalgae can ander conditions affrects the process of photosynthesis, and makes microalgae to produce secondary metabolites in the form of β 302 303 304 305 300 307 371 373 374 375 376 378 

384 385 386 387 Rendement of phycocyanin The rendement of phycocyanin protein content in Arthrospira was presented in Table 3. The pH condition of maintenance media can be affect of protein content in Arthrospira cells. The results of LSD on on the main factor of pH showed that the rendement of phycocyanin Arthrospira in treatment P2 was significantly higher compared to other treatments. Traifiquerations et al. (2017), the amount of Arthrospira biomass influences the high content of phycocyanis Table 1 showed that the highest of Arthrospira biomass was found in treatment P2. It showed that the highest Arthrospira biomass produced the highest rendement of phycocyanis (Table 3). The culture medium of Arthrospira PI of 8.5 produced the highest C-phycocyanis content (Istrated et al., 2010). Rahmawati et al. (2017) said that the higher of C-phycocyanis followed the higher of rendement of phycocyanis (Table 7). The culture medium of Arthrospira PI of 8.5 produced the higher of rendement of phycocyanis (Table 7). The culture medium of Arthrospira PI of 8.5 produced the higher of rendement of phycocyanis (Table 7). The culture medium of Arthrospira PI of 8.5 produced the higher of rendement of phycocyanis (Table 7). The culture medium of Arthrospira PI of 8.5 produced the higher of rendement of phycocyanis (Table 7). The culture medium of Arthrospira PI of 8.5 produced the higher of rendement of phycocyanis (Table 7). The culture medium of Arthrospira PI of 8.5 produced the higher of rendement of phycocyanis (Table 7). The phycocyanis followed the higher of rendement of phycocyanis (Table 7). The phycocyanis (Table 7) of 70.5 phycocyanis (Table 7). 

content (Istnaiel et al., 2010). Rahmawati et al. (2017) said that the higher of C-phycocyanin followed the higher of rendement of phycocyanin. LSD one showed that the main factor of salinity showed the rendement of phycocyanin in treatment S3 (salinity of 20 %) was significantly higher than other treatments. It is thought that the difference in salinity treatment has an impact on the external sumstiic pressure of Arthrospire cells which results in changes in cell compusition especially phycocyanin. Sodium will flow into the cell and cause the release of phycohilin (phycocyanin, phycocyanin and allophycocyanin) from PS II (Photosystem II) followed by activation of the protective mechanism. Arthrospire will produce carbohydrates to balance intracellular osmotic pressure and require more energy to remove sodium ions from cells. In this case it will produce ammonism assimilation causes inhibition of protein synthesis (Zhou et al., 2017). 397 

### Table 3. Physicsconin (%) yield in Arthrogensi dry hiemans at 8 days after it

Single Influence of pH (P)		Single Infl (LS	Main influence of pH (P)		
일상 같은 것 같은 것 같이 있는 것 같이 없다.	.51	82	53	54	(1.50 4.65-0.090)
P1 .	·7.881 *	6.783.4	9.441	36,3167.1	8.623 *
P2	9.657 /	10.906 *	11.347	10.423 *	10,583 =
P3	8.970	39,408.4	10.134 *	0.262 4	0.444 *
Main influence of Salmity (5) (LSD an=0.111)	8.836*	0.609 -	10.367 4	9,357 %	

The environment includes the availability of nutrients, pH, salinity, light and temperature can affect the growth and accumulation of hispigments from microalgae (Sharma and Tiwari, 2014). The condition of eulture media is able to influence the growth phase of *Arthrospiru*, causing changes in the composition and proportion of phycobilin (phycocertrin, phycocyanin and allophycocyanin) (Simeunovic et al., 2013). The results of the LSD are was showed that the rundament of phycocyanin. *Arthrospiru* on the interaction between factors in treatment PS2S was significantly higher than in other treatments, but it was not significantly different from treatment PS2S that and growth trate of *Arthrospiru*. The increasing salinity will cause maintenance media to be hypertonic towards cella and result in poor 400 407 

absorption of untrients by cells. These cells could reduce in protein and increase in carbohydrates from Arthrospira cells (Ravelonanileo et al., 2011). Production of phycocyanin was able to reach 12.4 % - 17.0% of biomass dry weight of Arthrospira outured in Zarmuk Medium (ZW) (Preite et al., 2018; Carcia-Lepez et al., 2020). There'are several factures that affect the rendement of phycocyanin includy temperature, extinction time, uniting rate, biomass, type of solvent and the ratio of biomass to the solvent (Tumfquerahme et al., 2018; Carcia-Lepez et al., 2020). There'are several factures that affect the rendement of phycocyanin includy temperature, extinction time, uniting rate, biomass, type of solvent and the ratio of biomass to the solvent (Tumfquerahme et al., 2016). The contant of phycocyanin in cyanohacteria increases when grown in low light intensity. Phycocyanin is a pigment associated with protein, polar and water soluble. The protein content of microalgae are influenced by arcitoromental conditions such as temperature, age of culture, light nonesity, phy, salinity, and nutricot limits (especially nitrogen). Nitrogen in an essential element needed for the synthesis of accessive pigments and solutorophyll. When miscroalgan are growing fact, it requires large monomis of nitrogen and could ecosime phycocyanin and an alternative assace of nitrogen for the production of bonness (Biain-Le et al., 2019). It must be optimized for biomase production and phycocyanin contents is lower than previous study, because of different media for culturing Anthrospira. The noticine from ZM (type analysis abbances) is more complete for growing and forming phycocyanin than assa water catful point media, supecially the true mineral in ZM.

### Reduction of Total Nitrogen and Phosphate Content The noncontage reduction of total nitrogen in the online modia was presented in Table 4. 427 428

Table 4. Robertion of total nitrogen content in driftengeine sidtum mediam (%)

Single Influence of pH (P)	2	Stagte In	Stain induces of pit		
N. Commences and the second	81	83	8.3	754	(LND au-0.049)
Pi	80.996*	82.256 -	83.767	81.897-	82.226 *
12	83.880 -	84.377 -0	63.420	84.857 -	84.853 *
123	8.2.940 <sup>http</sup>	81.143*	84,9991**	84.813 **	83.462 *
Main-Influence of Solinity	82,3990 *	83.856.5	84.712-	82.660 -	

The nuclearism for removing nitrogen in water is determined by several factors, suchaling bactarial activity (Gersberg et al., 1986), sptake by plants (Brezn, 1990) and evaporation (Sanchez-Monedrov et al., 2001). In Cyanobuckeria, nitrogen is a macromutirum that plays an important role in the formation of biochemical compounds such as muccias acids (DNA, BNA), amino acids (protein) and pignents (abloruphyll and phycocyanii) (Machani et al., 2014). The results of the LSD are on the reasin plf factor showed that the reduction in total nitrogen cluster in *introgen clusters* in *introgen clusters* in *introdeclusters* (abloruphyll and phycocyanii) (Machani et al., 2014). The results of the LSD are on the reasin plf factor showed that the reduction is total nitrogen cluster in *introgen clusters* in *introdeclusters* in *introdeclusters* (abloruphyll and Phycocyanii) (Machani et al., 2014). The results of the LSD was significantly higher than in invatments P1 and P3. This is due to the higher density of *Arthropatri* in culture medias is greater than others. Markou et al. (2014) showed that the higher density of *Arthropatris* is could be higher the absorption of matriants including nitrogen.

Markou et al. (2014) showad that the higher density of Arthrophys could be tagther the absorption or interation incoments infragent. The value of reducing total airrogen content in 53 treatment was significantly higher than for other treatments. This showed that treatment 53 caused Arthrophysic absorb introgen higher than other treatments in line with the high density and rendement of physocymin Arthrophysic absorb introgen higher than other treatments in line with the high density and rendement of physocymin Arthrophysic absorb introgen higher than other treatments in line with the high density and rendement of the study. Jabeen and Ahmad (2011) showed that salinity in culture media due to interactions between gH1 and animity factors showed that treatment P233 was significantly higher than other treatments. With the optimal conditions (P41 and aslimity is and rendement of physocymins found in treatment P233. The amount of airnet and physical density, growth rate and rendement of physocymins found in treatment P233. The amount of airnet and physical density growth of Choreeflo subgrowt and Arthrophysic Bayadi et al., 2010). This is because algue have the ability to absorb matternats and Arthrophysic Bayadi et al., 2010). This is because algue have the ability to absorb matternats and arthropen and Arthrophysic Bayadi et al., 2010). This is because algue have the ability to absorb matternats and and herbity aphrases is and physicogen of absorption, complexation, deposition and assimilation (between microbas and plant biomass) (Tammer et al., 1999).

	Single Influence of Salinity (S)				many and service and service of
single trainings of pri (r) -	: 36.8	19.2	76.0	3.4	state influence of pri (F
P1	20.500	71.007	73.900	22.333	21.875
12	74.667	74.006	74.667	72.007	7.4 (1993)
123	20.353	70.333	7.3,6457	22.667	21.750
Main influence of Salimity (5)	71.833	72.000	73.776	72.556	

454 455 450 The results of LSD sate on the main factor of pH showed that each treatment did not significantly affect the reduction of sphate content in Arthropping culture medium. Plants can only absorb phosphorus in the form of H\_PO<sub>4</sub> and HPO<sub>4</sub><sup>-2</sup> nh

free orthophosphate ions (Becquer et al., 2014). The orthophosphate content decreases with increasing media pH. Cerozi and Fitzsimmons (2010) showed the orthophosphate content increases in the pH range from 5.5 to 8.5 and decreases when pH 10. The fall in the orthophosphate content at pH 10 is due to an increase in calcium phosphate formation. The value of 457 458 459 400

pH 10. The fall in the orthophosphate content at pH 10 is due to an increase in calicium phosphate formation. The value of reducing the phosphate content in *Ardivaspira* culture mediam was presented in Table 5. The salinity facture, administration of different salinity in each treatment has no significant effect on reducing the phosphate content. Bassin et al. (2011) explained the reduction of phosphorus will be inhibited when a combination of Chromosphate content. Bassin et al. (2011) explained the reduction of phosphorus will be inhibited when a combination of Chromosphate content. Bassin et al. (2011) explained the reduction of phosphorus will be inhibited when a combination of Chromosphate content. Bassin et al. (2011) explained the reduction of phosphorus effect of differences in pH and salinity, each treatment had no significant effect on reducing phosphate content. Hun-Sheng et al. (1995) showed that the utilization of Dissolved Organic Phrsphorus (DOP) can be through active uptake into cells or by extracellular mineralization by phosphates enzymes. However, most DOP ompounds cannot be assimiliant directly with microalgae because they have been mineralized. Markou et al. (2014) stated that phosphorus is a macronutrisent that plays an important tole in the preparation of nucleic acids (RNA and DNA), phospholipids and energy-carrying molecules (ATP). The phosphorus content in plants is lower than Ca, N, and K (Sasaqi et al., 2018). Although the analysis of variance shows that the results are not significantly different, but the highest phosphate reduction still existed in treatment P2S3. 401 462 463 465 466 467 408 409 470

### 471 Morphology of Arthrospira in various pH and salinity media

Morphology of Arithmapian was offseted by increasing or decreasing physical or chemical factors in their culture media. Salinity and acidity value combination at this study dide't change the filament of Arthrospira significantly as being filament of Arthrospira under microscope with 100n magnification (Figure 7). The morphological form of Arthrospira were not different in all of pH and salinity treatments. The waste water catfish pond media could affected in the linierizing 472 473 474 475 476 filament. This study indicated that salinity and acidity of culture media didn't effect on various form filament, either straight and helical

477 478 479 straight and helical. The straight finaments were observed for *Arthrospiru* strains during two years of cultivation, and their presence in *Arthrospiru* sp. Nigrita C1 cultures was constant. The various morphological plasticity, greatly influenced by the growth stage and exogenous parameters, such as temperature and light intensity, was observed for *Arthrospiru* strains relatively rapid and also subsequent changes at the genetic level can be realized quickly. This means that we can easily find different genotypes in various stable, ecologically different habitate. Murphologically similar strains were cultured by a long time under uniform and stable coolditions (Komarek, 2016). But the morphological changes couldnot be effected by acidity and salimity of culture media. 480 481 482 483 484 485 480



429

P351	P382	P353	P3S4
Figure 7. Morphology of Arthrong Figures 7 is not clear.	should be redrive fo	media at several treatment of pH	and salimity
Conclusions Arthrospira that is collumn	I on waste media (observed	in liquid culture; indicated a	ome short and linear file

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Account operation in a contrast on watter metrica (observed in liquit) culture) indicated some short and linear filamental. Identified Arthroquien had a genetic distance of 6.2% between AF and AW isolatas. AF isolatas had a close relationship with Arthroquien hadroxic petil species originating from Japan (bocontrap value of 25%) which AW isolatas, form phylogenetic branches which are separated from AF indexts and Arthroppire plateous petit species originating from Japan (bocontrap value 85%). The earlish culture wastewater media at different pH and salinity affects the daman, growth rate and rendement of physice/yanin Arthroquiru pfortoatis. The highest density, growth rate and rendement of physice/yanin was in P233 treatment (pH N5  $\pm$  0.2 and alimity of 20%) which prevideed a maximum density of 0.867 g L<sup>-1</sup>, growth rate of 22.020% day<sup>-1</sup> and the rendement of physics/yanin of 11.334 %.

### ACKNOWLEDGMENT

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# Bukti konfirmasi submit revisi pertama, respon kepada reviewer, dan artikel yang diresubmit (28 November 2020)

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Ch	aracterization of Arthrospira platensis cultured in waste water of	Formatted: Fort: Italic
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which p	m sapan (bootstrap value of 85%). The best treatment in this study was P2S3 (pH 8.5 ± 0.2 with salinity 20 ppt roduced 0.807 grams maximum density, growth rate of 22.020 %-day <sup>-1</sup> and 11.347 me.e <sup>-1</sup> rendement s	ar ar
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15	INTRODUCTION	
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Basically, Arthroquira's morphology is characterized by trichomes that circular regularly (helical). However, abnormal morphology can also occur in Arthroquira as a circular shape that is irregular even linear. In some cultivation conditions, linear filaments can spontaneously return to the helix. However, there are significant differences in morphology, ultrastructure, physiology, huschemistry, and genetic characteristics between the original filament and the linear filament but not the difference between the original and the returned filament. Linearization in Arthroquira is a variation on the genetic level that can be caused by several environmental factors such as mutrition, light and content of water media for growth (Wang and Zhao 2005). According to Liu et al., (2016), DNA barcoding has developed as a reliable technology for identifying species based on variations in the sequence of standard DNA regions. This method is used successfully in a variety of biological applications including finding cryptic species, detecting invasive species, and identifying hor contrastructure to widely used to determine bacterial DNA characterization. Therefore, identifying here has been widely used to deturmine bacterial plan characterization of *Arthroquira* using the 165 rRNA gene needs to be done to get the characterization of *Arthroquira* that is cultured on technical fertilizer and waste media and determine the phylogenetic tree structure that has been recorded in GengBank. 5132334353657 58 39 60 61 62 63 64

Identification of Arthrospire using the 1685 rRNA gene neutro to the come to go use classesteration of arthrospire contrast and determine the phylogenetic tree structure that has been recorded in GengBank. Culture of Arthrospire (Spirwline) in Claries pool farming wastel water could have specific characterization for optimal phylogenetic tree structure that has been recorded in GengBank. Calture of Arthrospire (Spirwline) in Claries pool farming wastel water could have specific characterization for optimal phylogenetic in minorals, vitamins, and pignonen (Borowitzka et al., 2016; Vernes et al., 2016; Som color pignonts) that can be produced such as a nutritional value of 55-70% protein, -0.10% lipid, 20% carbohydrate, besides that can be produced such as physocryamin (blue pigmant) allophysocryamin (blue pigmant) (Sharma and Towari, 2011). Wernes et al., 2016; Vernes et al., 2016; Som color pigments that can be produced such as physocryamin (blue pigmant). Physocryamin (blue pigmant) (Sharma and Towari, 2011). Wernes et al., 2016; Vernes et al., 2017; Som color pigments that functions at an intioxidant (Prematyce and Linsanara, 2008; a source of food coloring, connetics, plaramaccuticalis and drags (Tang et al., 2020; Triwari & Tiwari, 2020), and inflammatory, anti-oxidative and anticurce (Liu et al., 2013). One of the factors that inflaence phycocynini levels is biomass (Tanfguruhmis et al., 2017). The pH and satisity of culture media can affect the biomass of Arthrospira (Cliferi, 1983). Marek et al., 1987; Planes et al., 2002), Ismaiel et al. (2016) showed that the diversity of the chemical composition of biomass is influenced by the pH of the prowth media. Value of pH and environmental factors, especially salinity, influence to eell compation (Hu, 2004). The optimal pH value of Arthrospira (Cliferi, 1983), Marek te al., 1987; Planes et al., 2002). The optimal pH value of first physical et al. 2015, Other and first physical et al. 2016, Strawari, 2005, The salinity ind physical et al., 2006, and salininy f 00 07 08 09 70 71 72 74 75 77 77 78 98 1823 84 85

### MATERIALS AND METHODS

MATERIALS AND METHODS
 driftrogplra collmered in agar media
 Material and sterilized using an nutsclave. Sterilized swamp water was then added with 0.02
 grams MgSQ2 fortilizer, CaCl; 0.004 gram, EDTA 0.008 gram, urea 0.03 gram. 2A (Stabilizer of Ammonipations)
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 EDTA 2.085/ms-summement) 20 grams / 100 ml (B solution (Calcium Nirrate 04.20%), Ammonipation sulfate
 9.41%, Mauganese sulfate 0.02%, Ammonium heptamolybdate 0.011/ms summonium 20 grams/100 ml water) and
 TSP [Triple Super Phocehattedetinning] 0.03 grams were fine homogeneous using magnetic stirters, Next, wasted water
 was striftized by an autoclave ther noiseld. Bactoagar was added to the technical fortilizer and wates solution to be
 homogenized and a magnetic attreer and then boiled using a hot place until all the ingredients dissolve and then autoclave
 ugain. The agar media was made with a pH of 7 and a salinity of 10 ont or lab gr1<sup>-1</sup> [Hindavani et al. 2019], (Where in the
 reference 1)

100 101 102 InformecTi (driftenprice cubivated in lapsid media was taken 100 µl using micropipatte and spread to the surface of a petri dish containing hactargar media by using a sterilized spreader roal. Petri dishes were wrapped in wrapping plastic and then given a lamp lighting (light intensity 2000-4000 lax) with a dark. light ratin = 0.24 hours. Arthrospira was observed every day until it grows blue green. After growing, driftenspira was re-cultured in agar media by the 4 quadrant scratch method. The cultures were used as isolate samplus for determining DNA barcodes. The biomass of driftenspira was isolated from the cultures were used as isolate samplus for determining DNA barcodes. The biomass of driftenspira was isolated from 103 104 105 108 107 nervial Spirulina, TopSpira East Jakarta, Indonesia, The the source of Aribourieu? The source of Arthrospira from Top5pina Spirulina East Jakarta. 108

What if

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DNA estructions DNA estructions was eserved out according to procedures in which there was a Presto TM Mini gDNA Bacteria Kat (Generald Biotech L.U.). DNA extraction consisted of several stager: sample perparation, lysis, purification, and precipitation or washing. The sample used was 0.15 grams of wat weight fits one extraction. Crement manualy...(Nhere a (Gen a tuferance?)

DNA amplification The process of DNA amplification using the PCR (Polymerase Chain Reaction) method was performed using 2 µ forward primers 607 (5°-CAGGCC TAA CAC ATU CAA GTC-3 ') and reverse primer 1387r (5°-GGG CGG WGT GT CAA GGC-3 ') (Marchesi et al., 1998). The total composition of the PCR mixture was 50 al which consisted of 25 µ1G Taq Green, 15 µ1 NPW (Nuclease Tree Water) and 5 µ1 Arthrospira DNA extraction template. DNA amplification was carried out in stager: the initiation cycle at 95°C for 5 minutes, followed by 30 denaturation cycles at 94 °C for 30 account mmealing at 55 °C for 30 seconds, then the extension stage at 72 °C for 1 minutes, and the final stage 72 °C for 7 minute (Lee et al., 2005). 117 118 119 120 121 123 124

### Electrophoresis

125 126 Electrophoresis was curried out using 1% agarose gel at 75 V for 35 minutes. Agarose that have been electrophoretic was immursed with a mixture of 10 µl diamond dye solution and 100 ml TAE 1x huffer solution for 30 minutes without exposure to light. The results were visualized through gel documentation by observing DNA migration using a transliminiator UV. 127 128

Gene Sequencing Arthrospirs DNA samples that were successfully amplified using PCR were then sequenced in the fragments of 16S rRNA gene. The amplified products were successfully amplified using PCR were then sequences obtained in the furn of fasti format were aligned using MECR A 0.0 software and then sploaded through the Basic Local Alignment Suech Tool (BLAST) program. BLAST was a program to warch for aud analyze the bornology of an organism's sequences, on the nchi.nln.nih.gov website so that its homology can be identified with other genus Arthrospira 108 rRNA gene sequences registered in the Gentlank database. The genetic distance and phylogenetic tree we separate were constructed using the Neighbor Joining (NI) method. The phylogenetic tree was constructed through the Maga 6.0 software application using the Neighbor Joining (NI) method. The phylogenetic tree was constructed using the Neighbor Joining (NI) method of the Maximum Composite Likelihood model and Substitutions to include d. Transitions + Transversions with 100ts boostrap. Meanwhile Arthrospira morphologicals form analysis were presented in the form of images and discussed descriptively by referring to the appropriate Interature. Optimization of pH and salmity for growing *Arthrospira* (CRD) conversions of the first factors with 3 treatments and 3 replications. The first factor was the difference of pH to culture media, including PI culture media pH 0.5 + 0.2, 2.92; culture modia pH 5.4 + 0.2 and 92; culture modia pH 10.5 + 0.2. The second factor was whe difference of salmity in culture media i.e. S1: salinity of 0.%s, S2: salinity of 10.%s, S3: salinity of 20.%s, and S4: salinity of 30.%s (What is the type of sali? Course sea sality. Calture memorations

Calture preparation The equipment used in this study was sterilized using 70% alcohol to minimize the contaminants that inhibit the productivity of *Arthrospira*. The containers used plastic bottles with opacity of 5.1, volume of 30 units. The plastic bottle were sterilized using a potassium permangante solution (2 mg. L<sup>+</sup>). Catfish culture wastewater obtained from catfish farming pseudo measuring 2 m s.1 m x 1 m, and high of water media was 20 cm (Figure 1). The density used in the posd was 320 fish, 400 L<sup>+</sup> with 150 grams fish<sup>+</sup>, maintained for 2 months by providing artificial food (protein 31%-33%), twice per day at satiation. Carfish culture wastewater was previously sterilized by boiling in an autoclave and then cooled, while the steril wastewater was twented with salinity. In treatments 51, 52, 53 and 54 were added with salt until salinity was obtained according to the treatment. The wastewater matha had a pH of 7.3, therefore there was an addition of HCL1 N at 0.75 mL<sup>+</sup>. In P1 resument to rusch a pH of 6.5, Meanvolds, in treatments P2 and P3, to ger a pH of 8.5 and pH 10.5, there was an addition of NaOH 8 N ns much as 0.07 ml L<sup>+</sup> and 0.45 ml L<sup>+</sup>.





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Figure 1. Catflah farming pond.

Figure 2. Arthrospics cultive

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Arthrospire previously used was cultured in carfish culture wastewater for culture stock with a density of 2 g L<sup>-1</sup>. The stock was taken as much as 400 mil is 3600 mil of carfish culture wastewater in accordance with treatment. Acration was used for agitation, the lighting using 30 wat TL lamps for 24 hours day<sup>-1</sup> during maintenance (Figure 2). Harvest of Bornass, 36 har are the componentia of wastewater<sup>2</sup> and phasehoru. 2.6 mg L<sup>-1</sup>, total memory and L<sup>-1</sup>. The part of the biomass was after exponential phase by filtering. The biomass was dried using an oven for 14 hours at 40 °C (Admined Phase by filtering: with modification). The dry biomass was used for the phycocyanin extraction process.

Phycos min extraction

The dty biomass was 0.04 g added by 1 ml of phosphate buffer pH 7, then homogenized and frazen in the freezer for 24 hours at a temperature of -4 °C. After 24 hours from the freezer, thaving process for 15 minutes. Samples were centrifugal for 30 minutes at 3000 rpm. After that, the sediment mind the supernaturit were separated. The resulting supernaturit was phycosyania which be analyzed using the Bennett and Bogorad method (1973).

The density of Aethrosphra biomass. Biomass density measurements were performed at each treatment and 3 replications every day at the same hour. The density of biomass was 1 ml of aample in each treatment with 3 replications, The 1 ml of simple into aluminam bowl. The animple and the aluminum bowl were weighed, then dried in the overs for 14 hours at 40 °C. The sample of water that had dried was weighed again. The dry biomass weight of Aethrospirat biomass was converted to g L<sup>1</sup>.



197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 Pigare 3. Dry biomass of Arthor 140.00 The growth rate of Arthrosptica can be calculated using the following formula according to Vonshak (1997): µ = In Nt - In No x 100%

 $\label{eq:generalized_states} \begin{array}{l} y_i = \mathrm{denly} \ generative (N_i + \mathrm{dayer}^2) \\ 1 = \mathrm{time} \ (\mathrm{dayer}) \ \mathrm{fixon} \ N_i = \mathrm{time} \ (\mathrm{dayer}^2) \\ N_i = \mathrm{density} \ \mathrm{ig} \ L^{-1}) \\ N_i = \mathrm{density} \ \mathrm{at} \ \mathrm{the} \ \mathrm{time} \ \mathrm{t} \ \mathrm{tg} \ L^{-1}) \end{array}$ 

Measurement of total nitrogen and phosphate content in culture enedia was carried out at the beginning and the first day after the peak phase of each treatment.

The measurement of pheocyanin refers to Bennett and Bogonal (1973). The absorbed supermatant was measured using a spectrophotometer at wavelengths of 615 nm and 652 nm.

# C-physiocyania (mg.ml.<sup>+</sup>) = \_\_\_OD 6151-0.474 (OD 652).

Rendement of phycocyanin (mg g<sup>2</sup>) = <u>C-phycocyanin & V</u> DB

Rendement percentage of phycocyanin (%) ~ Randement of phycocyanin (mg.g.<sup>1</sup>) x 100%. Note:

C-phycocyania = C-phycocyania consemination (mg. mL.<sup>2</sup>) V = Solvent Volume (ml) DNI = Dry Biomasa (0.04 g) 0.474 and 5.34 = coefficient of extinction (Bennett and Biogorad, 1973)

The results were submitted to simple analysis of variance tests (ANOVA) (p <0.05) and in the case of significant differences, the means were compared by the Least Significant Differences test (p<0.05). Т

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### RESULTS AND DISCUSSION

### 231 Morphology of Arthrospira

Arthropping was caltivated using two different fertilizer media namely technical fertilizer and waste media. The morphology of commercial Arthropping before fertilizer treatment was presented in Figure 4. 232 233



interspiral ; AF= commercial striterospira alia) 40x magnification 248 Figure 4. Morphological identification results of Arthrue cultured with technical fartilizer multir, AW- commercial throuping isolate (CA =Commercial drillows relat drillowgning cultured with wante media) 4

The results of the identification of isolates showed that the isolate had a twisted filament shape resembling a spiral (helicat). Based on Davin's identification book (1955), it is known that the isolate used in the study was *Solvalian* (*Arthrospira*) platents. *Arthrospira*: is cyanohecteria belonging to the order Oscillatoriales which has a filament (trichome) that resembles a spiral (helicat). Based on Davin's identification book (1955), it is known that the isolate used in the study was *Solvalian* (*Arthrospira*) platents. *Arthrospira*: is cyanohecteria belonging to the order Oscillatoriales which has a filament (trichome) that resembles a spiral (helicat). Based on Davin's identification from the air (*Argent et al.*) (1998). Heterocyst cells are special thick-walled cells that play a role in nitrogen fixation from the air (*Argent et al.*) (1998). Heterocyst cells are special thick-walled cells that play a role in nitrogen fixation from the air (*Argent et al.*) (1998). Heterocyst cells are special thick-walled cells that play a role in different media had several linear/straingly morphologics. Based on Figure 2, *Arthrospira*: which was cultured on technical fertilizer media has a longer and spiraling morphological form compared to another cultured on waste media. Their filaments have more linear morphological form, some spirals but not too long. According to Astiani et al (2016), *Arthrospira* ignorthic and high light intensity. In this study, solates were cultured with the same light intensity of 2000-3000 has with a light dark rule of 92.4 hours. Linear filaments into of 0:24 hours. Size related the hours in *Arthrospira* is a variation on the genetic level that can be caused by environmental factors such as lack of nutrition and high light intensity. In this study, solates were cultured with the same light intensity of 2000-3000 has with a light dark rule of 92.4 hours. Linear filaments in AW have a lower metabolic rate compared to helical filaments. This is one of the adaptive mechanisms for *Ar* 250 251 253 254 255 256 257 258 259

Tabel, I. The results of the BLASTn analysis of driftrospirst samples cultivated to tachnical fartilizer and waste mediants with data a

Description	Identity (76)	Access ende	Sample wright
Arthrouptina (fortilizor)	and the second second second		
Erthrouging platownia petH	100	AB113346	Aspan
Spirulina platennix CCC 478	90.48	JX014313.1	India
Spirulina platentix cyati	94.4	E>49531.1	Japan

Arthrospics platonis PCC 7343 Arthrospics maxima EEW2	90,12 74,4	15833264.1 14Q068225	USA Australia
Arthroughna Datonic putt Arthroughna Datonic putt Arthroughna Datonic DEC 452	94,3	D49531.1 MG012588.1	Japan
Spinding plannsis CCC 478	74.4	120014313-1	India
Arthropping maximu str. Lefevre 1963/M-132-1	73.3	F37984-12	Vessenantta
Arthroughing maxima EEW2	72,2	HQ088225	Australia

365

Phylogenetic Tree The results of the 16S rRNA encoding gene sequences from AF and AW isolates were traced to other *Arthrospiru* isolates present in GenBank through the BLAST program. The results of the BLASTs analysis of *Arthrospiru* analysis collivated in sechnical fertilizer and waste media with data in Genbank are presented in Table 1. Table 1 results of the BLAST analysis show the closeness between AF and AW isolates with other isolates in conflants. It about that *arthrospiru* isolates and *Arthrospiru* awate isolates have the closest homology to *Arthrospiru* planeado peth species from Japan with percentage values respectively 100% and 94.3%.



Figure 5. Phylogenetic analysis with 1600 basisting AW (definespire cultured in waste water readiat and AF (definespire cultured in waste)

Bertilizer madial
 Genetic distance was used to see kinship relationships from *Arthrospira* both AF and AW samples with sequential data from Genbank. AF isolate indicated a genetic distance of 0.008 with AW isolates. Atward AF isolates showed the lowest instance respectively 0.089 million of *Arthrospira* platnamic prefit from Japan. Analysis based on greetic distance on 0.006 (0.4%) meaning that there are intraspecte variations in the ample caused by mutations.
 Phylogenetic tree *Arthrospira* isolates from technical fartilizer and waste media were presented in Figure 5. The phylogenetic tree from Argan. Analysis based on greetic intraspect was 0.006 (0.4%) meaning that there are intraspectes variations in the ample caused by mutatices.
 Phylogenetic tree *Arthrospira* isolates from technical fartilizer and waste media were presented in Figure 5. The phylogenetic tree form Argan. Arg Avis based on greetic distance was separate tree is a strategies of the second presented was separate with several other *Arthrospira* isolates through relationships between enganisms or population characters based on their evolutionary history. The result of phylogenetic tree construction showed that both samples formed branches with educite. Phylogenetic tree form Ar AW isolates and AW isolates and AW isolates and the second present was separate with several other *Arthrospira* plates of the level of confidence in grouping tecostamy value is considered high become according to Hall (2001), a clade cam be treested with a beotrarp value of 90%. In addition, Hillis and Bull (1993) state the broach of AF isolates and *Arthrospira* platencies appeting malysis with values of 70% or higher influence is reliable grouping. The AW isolates and Arthrospiral additioned and malysis. Genetically, they had former with a bottime value of a separate brach of AF isolates and a different strate from Articolate greeps. Ballet et with a boottime value of 19%. So et al. (2004) estated that *Sphrathar* 

Density an The bi

Density and Growth Rate of Arthrospira collured in numerouter of catfith farming. The biomass of Arthrospira displayed mechanism of adaptation in culture media. The wastewater media could make different charateristic of growth as like as filament. The masimum densities of Arthrospire cultured in waited water were achieved on a different day. The daily density of Arthrospire during culture can be seen in Figure 6. differ



The graph presented in Figure 6, it show that in the culture period from day 1 to day 2, Arthropping in each treatment The graph presented in Figure 6, it show that in the culture period from day 1 to day 2. Arthropira in each treatment experienced slow growth, because the cells were still adapting to their new environment. The exponential phase for the treatment of P2S2, P2S3, and P3S3 last from day 1 to day 8 of the culture period. In the treatment of P2S1, P2S4, P3S1 and P3S2 take place from  $2^{nd}$  until  $8^{th}$  day of the culture period. While the treatment of P1S1, P1S2, P1S3, P1S4, and P3S4 lasted from  $3^{nd} = 8^{th}$  day of the culture period. The decreasing Arthroppira density for the treatment of e2S1, P2S4, P3S1 and P3S2 take place from  $2^{nd}$  until  $8^{th}$  day of the culture period. While the treatment of P1S1, P1S2, P1S3, P1S4, and P3S4 lasted from  $3^{nd} = 8^{th}$  day of the culture, period. The decreasing Arthroppira density for the treatment occurred from the day 9 to the day 11 of the culture. Lesiman et al. (2019) explained that the adaptation plase lasts from day 0 to day 1, while the exponential plase occurs from dny 1 to day 7, and experiences a stationary phase from day 7 to day 9 then enters the deash phase after the 7<sup>th</sup> day and 9<sup>th</sup> day. The decrease of density could because of reducing the natrients in the culture media. Soni et al. (2019), the concentration of numeration the day between 5 – 8 days after culturing. The mean of maximum density could be 0.433.04.057 et 1<sup>-5</sup> day in culture media. 337 338 

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The maximum density of Arthrospira could be achieved on different day, between 5 – 8 days after culturing. The mean of maximum density could be 0.433 - 0.867 g L<sup>-1</sup> of dry biomass which cultured in cuffish rearing waste water. The maximum cellular density of A, plactorsis which cultured in the fash rearing waste water. The maximum cellular density of A and the culture of the fash rearing waste water. The maximum cellular density of A and the culture of the fash rearing waste water. The maximum productivity of 0.03 g L<sup>-1</sup> day<sup>-1</sup> (Nogueira et al. 2018). The catfish rearing pond waste water has high potential as cultivation media for Arthrospira production. The analysis of variance showed that differences in pH, salinity and interaction between factors (pH and salinity) significantly affect the maximum density and growth rate of Arthrospira planetsci. The results of the LSD  $_{0.01}$  tractions of differences in pH, salinity and interaction between factors (pH and salinity) significantly affect the maximum density and growth rate of Arthrospira planetsci. The results of the LSD  $_{0.01}$  tractors of differences in pH, density and growth rate of Arthrospira platents is not been and the trans the maximum behavior of Arthrospira platents (pH 10.5 ± 0.2). According to Ismaiel (2016), the highest biomass of Arthrospira platents (pH 10.5 ± 0.2). According to Ismaiel (2016), the highest biomass of Arthrospira platents (pH 10.5 ± 0.5). Although Arthrospira platents is an other set as a wide pH range, a pH range farther from its optimal pH can reduce its growth rate. A low growth rate will also cause low biomass production. Furthermore, the different salinity treatment factors showed that the maximum density and growth rate in treatment S3. 347 348 351 352 354 355

While the density and growth rate between \$1 and \$4 treatments showed that the maximum density and growth rate in treatment \$3. Furthermore, the different salinity treatment factors showed that the maximum density and growth rate in treatment \$3. (salinity of 20 %s) were significantly higher compared to \$1 (salinity of 0 %s), \$32 (salinity of 10 %s) and \$4 (salinity of 30 %s) treatments. The \$1 and \$4 treatments were not significant and were the treatments that produced the lowest density compared to other treatments. Table 1 and Table 2 showed that the highest density and daily growth rate was found in the \$3 (salinity of 20 %s) treatment. This is supported by the results of Koulgardi et al. (2015), that *Arthreagniru plateosis* cultured on Conway media was able to produce the highest density of 912.07 m.L<sup>2</sup>. cells at a salinity of 20 %s. While the density and growth rate between \$1 and \$4 treatments showed no significant difference. This is because the salinity of 0-30 %s is still within the range of salinity that can be tolerated by *Arthrospiru*. Ugfly et al. (2015) said that *Arthreospiru plateosis* is one of the species of *Cyumobacteria* that can grow in an euryhaline environment. 358 

Table 1. Maximum density of Arthrogetica platensis (g L 2)

Single Influence of pH (P)	(1.5D use = 0.107)				Main tuffmence of pH (P)
	-S1(0.5a)	52 (10 %)	53 (20 No)	S4 (30 %)	(ESD (.8-0.053)
P1 (pH 6.5)	0.433 *	0.633 *	0.767 004	0.467 *	0.575 *
P2 (pH 8.5)	0.767 ohr	0.833 **	0.867	0.800 ***	0.817 4
P3 (pH 10.5)	0.667 **	0.733 hole	0.767 -met	0.700 htt	0.717*
Main influence of Salinity (\$1/1 \$13 parts 0.067)	0.622 *	0.733 +	0.900 *	0.656 *	

Table 1. The arrestly rat	e of definitions and	interests cultured in pH	usual webbrooky to contracted the short	v

Single Influence of pH (P)		Single Influ (LSD	ence of Salinity (S e.m = 3.224)	9	Muin influence of pH (P)
	81(0.%)	S2 (10 %)	N3 (20 %)	54 (20 Ter)	(LSD car+1.012)
P1 (plt 6.5) P2 (plt 6.5) P3 (plt 10.5)	14.333 18.343 <sup>bed</sup> 20.023 <sup>de</sup>	18.659 at 20.570 ± 19.527*	10.102 -* 22.026 * 20.823 *	15.348 + 20.723 # 16.417 #	16.383 * 20:416 * 19:347 *
Main auflicence of Solinity (\$1(LSD ===1.051)	17.566*	19.585*	20.614*	16.829*	

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uty 10:20 and 107 ath rate. How is this given growth rate?

of sufinity between fresh water (0 opt), of Arthroppica.

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The interaction between pH and salinity factors showed that the density and growth rate in treatment P253 (pH 8.5 ± 0.2 and salinity of 20 %) were significantly different from treatments each as P153, P254, P352, P254, and P353. While in the growth rate treatment P253 was not significantly different from P163, P252, P254, P352, P254, and P353. While in the growth rate treatment P253 was not significantly different from P163, P252, P254, P352, and P353. While in the growth rate treatment P253 was not significantly different from P163, P252, P254, P352 and treatment P353. Table 1 and Table 2 show that treatment P2 (pH 8.5 ± 0.2) is more dominant causing higher density and growth rate of Arthrospita plateotic density when combined by the treatment S1 (salinity 20 ppl). The treatment of P1, P2, and P3 (pH 0.5 ± 0.2) movide the highest density when combined by the treatment S1 (salinity 20 ppl). The treatment of P1, P2, and P3 (pH 0.5 ± 0.2) movide the highest density when combined by the treatment S1 (salinity 20 ppl). The treatment of P1, P2, and P3 (pH 0.5 ± 0.2) movide the fortwopira plateotic distance from distance is salinity of 0 %. 10 %. 30 %, and 30 % ob sill support the growth of drivtopira plateotic distance for the probability of the second distance of the probability of the probability of the second distance distance will increase (Hoyd, 1990). Prasad (2018) showed that growth of *Arthrospira* actual the distance of the second are treated as the second distance distance or state distance or the second distance distance or second distance distance or second distance distance or second distance distance or second distance distance will increase distance distance or the second distance distance distance distance distance distance dista 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401

Reinforment of phycocyanities The rendement of Arthrospira was presented in Table 3. The pH condition of maintenance media can be affect of protein curimit in Arthrospira cells. The results of LSD see on the main factor of pH showed that the rendement of phycocyanin Arthrospira in treatment P2 was significantly higher compared to other treatments. Taufiquanthesis et al. (2017), the amount of Arthrospira biomass influences the high content of phycocyanin. Table 1 showed that the highest Arthrospira biomass was found in treatment P2. It showed that the highest Arthrospira biomass produced the highest rendement of 8.5 produced the highest rendement (Isnaie) and 10.6 produced the highest of rendement (Isnaie) et al., 2010). Rahmawati et al. (2017) said that the higher of C-phycocyanin followed the highest of rendement (Ismaiel et al., 2010). Rahmawati et al. (2017) sint une use second and phycocyanin in treatment S3 (salinity of 20 1.SD <sub>bat</sub> showed that the main factor of salinity showed the rendement of phycocyanin in treatment S3 (salinity of 20 https://www.commun.com/salinity.com/salin

411 1.50 satisfies the weat that the main factor of satisfies showed the rendement of physocyamin in treatment 55 (satisfies) of 20 56) was significantly higher than other treatments. It is thought that the difference in salinity treatments has an impact on the external consotic pressure of Arthropyton cells which results in charges in cell composition especially physocyania from PS II (Photosystem UI) and stop the electrons transporting to PS I (Photosystem I) followed by activation of the protective mechanism. Arthropyton will produce carbohydrates to balance intracellular isomotic pressure and require more energy to remove solution in from relative in this case it will produce annonium assimilation causes inhibition of protein synthesis (2bou et al., 2017). 412 413 414 415 415 416 417 418 419

### 420 Table 3. Physicscennin (%) yield in Arthrosovice dry biomass at 8 days after in-

Singly Influence of pH (P)	Single Influence of Salinity (5) (LSD <sub>nat</sub> = 0, (94)				Main influence of pH (P)
	51	52	6.3	55-6	(LSD 6.02-0.3996)
P1	7.881 *	8.783	9.441.1	8.387 5	8.623 *
P2	9.657.1	10.966 *	11.347 1	10.423 =	10.583 +
P3	8.970 *	19.408 <sup>-4</sup>	10.134 7	9.263 *	9.444 *
Main influence of Salinity (5) (L5D aar=0.111)	8.3676 *	0,000+	10.367 4	9.357 5	

422 423 424 425

 

 (S)(1.5D surved.11)
 8.86°
 9.89°
 10.30°
 9.33°

 The environment includes the availability of nutrients, pH, salinity, light and temperature can affect the growth and nutrients in phase of Arthrospira, causing changes in the composition and proportion of phycocyanin and allophycocyanin) (Simeunovic et al., 2013). The results of the LSD are was showed that the rendement of phycocyanin and allophycocyanin (Simeunovic et al., 2013). The results of the LSD are was showed that the rendement of phycocyanin and allophycocyanin (Simeunovic et al., 2013). The results of the LSD are was showed that the rendement of phycocyanin and allophycocyanin (Simeunovic et al., 2013). The results of the LSD are was showed that the rendement of the phycocyanin is net significantly different from treatment P2S3. This showed from the density and growth rate of Arthrospira on the interaction between factors in treatment P2S3. The showed from Arthrospira cells (Bavelonandro et al., 2011).

 Werelowed the interaction of phycocyanin was able to reach 12.4 % - 17.6% of biomass dry weight of Arthrospira cultured in Zarroak Medium (ZM) (Prates et al., 2018). Garcin-Lopez et al., 2020). There are several factors that affect the rendement of phycocyanin is a pigment associated with protein, polar and water soluble. The protein content of microalgae reinfluenced by environmental conditions such as temperature, get called the availability, Phycocyanin is an eigenstial content of phycocyanin is of accessory pigments and children (Bave). When wirroalgae are growing fast, it requires large amounts of althrospira della conduction of phycocyanin is an essential clement needed for the ayuthesis of accessory pigments and childrospira. The higher content is lower than previous study, because of different form fast, phycocyanin and phycocyanin and phycocyanin is of phycocyanin and phycocyanin and phycocya 420 427 428 430 431 432 433 434 435 430 437 438 439 440 441 442

444 445 446 447 Reduction of Total Nitrogen and Phosphate Content The percentage reduction of total nitrogen in the culture media was presented in Table 4.

### Table 4. Reduction of total nitrogen content in driferosories culture mediaen (%) Single Influence of pill (P) Single Influence of Salitation (1.200 met = 1.290) 51 52 53 P1 \$19,900 \* \$12,250 \* \$10,707 \*\*\* \$10,707 \*\*\* \$10,707 \*\*\* Main influence of pH (P) (LSD ant=0.045) 82 226 \* \$1 \$0,990 \* 54 11.107 = 84.033\* 83.810<sup>±0</sup> 82.940<sup>to1</sup> 85.420 <sup>4</sup> 84.950 <sup>41</sup> 64,377.\*\* 81,143 \* 84.837 <sup>---</sup> 84.813 <sup>---</sup> Mai 87.500 -83.856 7 84.712 82.603 \* (S) (LSD 0.0+0.745)

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The mechanism for removing nitrogen in water is determined by serveral factors, including bacterial activity (Gersherg et al., 1986), uptake by plants (Breen, 1990) and evaporation (Sanchez-Monodeco et al., 2001). In Cyanobacteria, nitrogen is a macronutrient that plays an important role in the formation of biochemical compounds such as nucleic acids (DNA, RNA), amino acids (protein) and pigments (chlorophyll and phycocyanin) (Markou et al., 2014). The results of the LSD can on the main pH factor showed that the reduction in total nitrogen content in *Arthrospira* culture media in treatments P1 and P3. This is due to the higher density of *Arthrospira* in treatment p2 compared to other treatments, so that the utilization of nitrogen W *Arthrospira* could be higher than a others. Markou et al. (2014) showed that the higher density of *Arthrospira* could be higher the absorption of nutrisents including nitrouen. 449 450 451 452 453 454 455 455 455 nitrogen

nitrogen. The value of reducing total nitrogen content in S3 treatment was significantly higher than for other treatments. This showed that treatment S3 caused Archrospira to absorb nitrogen higher than other treatments in line with the high density and rendement of phycacyanin Archrospira to absorb nitrogen higher than other treatments in line with the high density and rendement of phycacyanin Archrospira obtained in this study. Jabeen and Ahmad (2011) showed that salinity in culture media influences nitrogen absorption and protein biosynthesis. Reduction of total nitrogen content in Arthroppira culture media due to interactions between pH and salinity factors showed that treatment P253 was significantly higher than other treatments. With the optimal conditions (pH and salinity), Arthroppira is able to make maximum use of nitrogen. This can be seen from the highest density, growth rate and rendement of phycocyanin found in treatment P253. The 458 459 460 401 402 403 404

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549 550 unsumt of nitrute and phosphate decreases with increasing growth of *Chlorella vulgaris* and *Arthrospira platensis* (Sayadi et al., 2016). This is because algae have the ability to absorb nutrients such as nitrogen and phosphate are use to carry out photosynthesis and protein protein production. Reduction of phosphorus content in waters is influenced by the process of absorption, complexation, deposition and assimilation (between microbes and plant biomass) (Tanner et al., 1999).

Table 5. Reduction of phosphate content in dethroughly culture madinit (%)							
Alternative Bauertenannen und beste derte		Single Influen	Barrier to the second of other sets				
single influence at pit (P) -	NT.	82	83	29.4	- stant ontoinee of pri (r)		
71	70,500	71.667	73.000	72.333	71.875		
72	74.667	74.000	74.067	72.667	74.000		
P3	70.333	70.333	73.667	72.667	71.750		
Main influence of Salinity (S)	71.833	72.000	73.77m	72.550			

The results of LSD and on the main factor of pH showed that each treatment did not significantly affect the reduction of phosphate content in *Arthrophysica* culture medium. Plants can only absorb phosphare in the form of  $H_2PO_4$  and  $HPO_4^{-2}$  free arthophosphate content in *Arthrophysica* culture medium. Plants can only absorb phosphare in the form of  $H_2PO_4$  and  $HPO_4^{-2}$  free arthophosphate content in *Arthrophysica* culture medium. Plants can only absorb phosphare in the form of  $H_2PO_4$  and  $HPO_4^{-2}$  free arthophosphate content in content increasing media pH. Cercoir and Fitzsimmons (2016) showed the orthophosphate content increases in the pH range from 5.5 to 8.5 and decrease when pH 10. The fall in the orthophosphate content is plut 10 is due to an increase in calcium phosphate formation. The value of reducing the phosphate content in *Arthrophysica* culture medium was presented in Table 5. The salinity factor, administration of different salinity in each treatment has no significant effect on reducing the phosphate content in *Arthrophysica* culture medium was presented in Table 5. The salinity factor, administration of different salinity in each treatment has no significant effect on reducing the phosphate cuntent. Bassin et al. (2011) explained the reduction of phosphates util be inhibited when a combination of C1 and nitrite and C1 concentration more than 2.5 g L<sup>-1</sup>. The interaction effect of differences in pH and salinity, each treatment had no significant effect on reducing phosphate content. Has-Sheng et al. (1995) showed that the unitization of Dissolved Organic Theophores. However, most DOP compound curture be assimilated directly with microalgue became they have been mineralized. Markon et al. (2014) stated that phosphate is an macroattrient that plays an important role in the preparation of macleic ascids (RNA and DNA), phaspholing is and energy-carrying molecules (ATP). The phosphate content in plants is lower than Ca, N, and K (Sasaqi et al., 2018). Although the analysis of v

The next inguiricancy different, but the lighter phosphate reduction suit econed in reclament P255.
Morphology of Arthrospira in various pll and salinity media
Morphology of Arthrospira was affected by increasing or decreasing physical or chemical factors in their culture media. Salinity and acidity value combination at this study didn's change the filament of Arthrospira significantly as their filament of Arthrospira under microscope with 100x magnificantly increasing or document of Arthrospira significantly as their filament. The waste water caffah point media could affected in the linierizing filament. This study indicated that admitty and acidity of culture media didn't effect on various form filament, either straight filaments were observed for Arthrospira strains during two years of cultivation, and their presence in Arthrospira presences in a temperature and light intensity, was observed for Arthrospira significant in a temperature media didn't observed for Arthrospira significant y and acidity influences and their presence in Arthrospira and exogenous parameters, such as temperature and light intensity, was observed for Arthrospira strains (Papapanagiotou and Okclis, 2019). There are indications that adaptability to change in environmental conditions is relatively input and allos advacuent changes at the genetic level can be realized quickly. This means that we can easily indifferent genotypes in various stable, ecologically different habitats. Morphological changes couldnot be effected by acidity and salinity of culture media.



1.57	P252	P283	P254
	type	1 Jay	R
1. M	1 desta	2.110	the
		5 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	

Figure 7. Mornhology of Arthoughing in catfiely up whether have 110 ho too Figures 7 is not clear, should be redrawn for high quality. We are very sorry for not having avoid quality images. We hone that the pictu Arthrospira linierizing filament, morphologically, re (figure T) are still enough fig determined

### Conclusions

Conclusions Arthrospirm that is cultured on waste media (observed in liquid culture) indicated some short and linear filaments. Identified Arthrospira had a genetic distance of 6.8% between AF and AW isolates. AF isolates had a close relationship with Arthrospira plateasis path apecies originating from Japan (bootstrap value of 95%), while AW isolates form phylogenetic branches which are separated from AF isolates and Arthrospira plateasis peth apecies originating from Japan (bootstrap value 85%). The earlish culture wastewater media at different pH and anlinity affects the density, growth rate and rendement of phycocyanin Arthrospira plateasis. The highest density, growth rate and rendement of phycocyanin was in P283 treatment (pH 8.5 ± 0.2 and salinity of 20 %) which produced a maximum density of 0.867 g L<sup>-1</sup>, growth rate of 22.020% day<sup>-1</sup> and the rendement of phycocyanin of 11.334 %. 538 539 540 541 542 54) 544

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# Characterization of Arthrospira platensis cultured in waste water of Clarias catfish farming media: DNA barcode, helical form, growth, and phycocyanin

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<sup>a</sup>Department of Soil Science, Faculty of Agriculture, Sriwijaya University. Jl. Palembang Prabumulih Km 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia. Tel/Fax, +62-711-580059, \*email: mariniwijayanti@fp.unsri.ac.id

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Abstract. Wijayanti M, Syafudin M, Yulisman, Nurianti Y, Hidayani A, Gofar N. 2020. Characterization of Arthrospira platensis cultured in waste water of Clarias catfish farming media: DNA barcode, helical form, growth, and phycocyanin. Biodiversitas 21: xxxx. Arthrospira production technology in catfish waste media can be an alternative to reduce environmental pollution. However, some environmental factors such as nutrition, light and water content can influence characterization of Arthrospira at the genetic and physiologic level. Arthrospira platensis is one of the phycocyanin-producing cyanobacteria and can be cultured using catfish culture wastewater. Water quality especially pH and salinity can effect of growth rate and rendement of phycocyanin from Arthrospira platensis. This study aimed to identify the species and morphological forms of Arthrospira cultured using technical fertilizer and waste media, as well as to know the phylogenetic trees between species in this study and the GeneBank based on the 16S rRNA gene, and determine the optimum of pH and salinity required in the medium of catfish culture wastewater to phycocyanin maximum production of Arthrospira. The optimation of pH and salinity method used Completely Randomized Design (CRD) factorial with 2 factors consisting of the first factor with 3 treatments and the second factor with 4 treatments and 3 replications. The first factor was pH of culture medium i.e. pH 6.5 ± 0.2, pH 8.5 ± 0.2 and pH 10.5 ± 0.2. The second factor was salinity of culture medium, that were salinity 0 ppt(Parts per thousand (10, 10 ppt, 20 ppt and 30 ppt. Parameters observed in Arthrospira include density, growth rate, rendement of phycocyanin, and decreased of total nitrogen and phosphate content in culture media. The results showed that morphology Arthrospira cultured on technical fertilizer media (AF) had a longer and helix filament compared to Arthrospira cultured on waste media (AW) which showed several linear and shorter filaments. Both samples have a genetic distance of 0.068 (6.8%). Phylogenetic trees indicated that AF had a close relationship with Arthrospira platensis petH from Japan (bootstrap value 95%). While AW formed a separate sub cluster of AF isolates and Arthrospira platensis petH from Japan (bootstrap value of 85%). The best treatment in this study was P2S3 (pH  $8.5 \pm 0.2$ with salinity 20 ppt), which produced 0.867 grams maximum density, growth rate of 22.026 %, day1 and 11.347 mg.g1 rendement of phycocyanin.

Keywords: catfish culture wastewater, DNA barcode, pH, phycocyanin, phylogenetic analysis, salinity, Spirulina(Arthrospira),16S rRNA

### INTRODUCTION

Arthrospira is a genus of cyanobacterial microalgae, commonly known under the taxonomically incorrect brand name 'Spirulina' (Papapanagiotou & Gkelis, 2019). The cyanobacterial genus Arthrospira Stizenberger ex Gomont 1892 contains at present 23 species, along with 12 infraspecific taxa (Guiry & Guiry, 2010). They have variety characteristic of molecular, morphology, and physiology that based on polyphasic approach. Various genotypes are adaptable to various specialized ecosystems. The combination of different methods should be based on molecular sequencing as the basic approach, to which must be added other criteria (morphological, ecological) if they are available and which are distinct and recognizable in cyanobacterial populations (Komarek, 2016). A polyphasic approach to include all the criteria obtained from morphological, biochemical, molecular studies, and

phylogenetic to understand cyanobacterial classification as like as Arthrospira classification (Komárek, 2018).

Recent studies have shown that Arthrospira can be used for treating wastewater, including effluent from fish culture, because the biomass can metabolize the nutrients and remove the pollutant from aquaculture effluent efficiently (Zhang et al., 2019). Industrial and processing wastes and by-products for culturing Spirulina (Arthrospira) are also being considered as alternative culture media, as like as aquaculture waste water (Ragaza et al. 2020, Wijavanti et al. 2018, Widvantoro et al., 2018). Aquaculture could apply an integrated strategy of simultaneously treating aquaculture effluent while producing the biomass to supplement fish diets. The nutrient composition in their biomass depends on their environmental factor for growing biomass. Their character could be different with the various media for growth.

Basically, Arthrospira's morphology is characterized by trichomes that circular regularly (helical). However, abnormal morphology can also occur in Arthrospira as a circular shape that is irregular even linear. In some cultivation conditions, linear filaments can spontaneously return to the helix. However, there are significant differences in morphology, ultrastructure, physiology, biochemistry, and genetic characteristics between the original filament and the linear filament but not the difference between the original and the returned filament. Linearization in Arthrospira is a variation on the genetic level that can be caused by several environmental factors such as nutrition, light and content of water media for growth (Wang and Zhao 2005). According to Liu et al., (2016), DNA barcoding has developed as a reliable technology for identifying species based on variations in the sequence of standard DNA regions. This method is used successfully in a variety of biological applications including finding cryptic species, detecting invasive species, and identifying plants. DNA barcoding is a simple short genome sequence amplified via PCR using appropriate primers (Adamowicz, 2015). DNA barcoding using the 16S rRNA gene has been widely used to determine bacterial DNA characterization. Therefore, identification of Arthrospira using the 16S rRNA gene needs to be done to get the characterization of Arthrospira that is cultured on technical fertilizer and waste media and determine the phylogenetic tree structure that has been recorded in GeneBank.

Culture of Arthrospira (Spirulina) in Clarias pond farming wasted water could have specific characterization for optimal pH value and salinity. Their adaptation to grow in organic waste water makes change in bioactive and important compound production. Their biomass has a nutritional value of 55-70% protein, 6-10% lipid, 20% carbohydrate, besides being rich in minerals, vitamins, and pigments (Borowitzka et al., 2016; Vernes et al., 2015). Some color pigments that can be produced such as phycocyanin (blue pigment), allophycocyanin (blue-green) and phycoerythrin (red pigment) (Sharma and Tiwari, 2011; Vernes et al., 2015). Phycocyanin is pigment in Arthrospira which has functions as an antioxidant (Pirenantyo and Limantara, 2008), a source of food coloring, cosmetics, pharmaceuticals and drugs (Tang et al,. 2020; Tiwari & Tiwari, 2020), anti-inflammatory, antioxidative and anticancer (Liu et al., 2013). One of the factors that influence phycocyanin levels is biomass (Taufiqurrahmi et al., 2017). The pH and salinity of culture media can affect the biomass of Arthrospira (Ciferri, 1983: Marek et al., 1987; Planes et al., 2002). Ismaiel et al. (2016) showed that the diversity of the chemical composition of biomass is influenced by the pH of the growth media. Value of pH and environmental factors, especially salinity, influence the productivity of cell biomass, photosynthesis, shape, and flow of cellular metabolic activity that affect the dynamics of cell composition (Hu, 2004). The optimal pH value for growth of Arthrospira sp is 7-10.5 (Hariyati, 2008), and salinity from 15-30 ‰ (Thajuddin and Subramanian, 2005). The

salinity and pH value of Arthrospira culture media have been known to affect the morphology of the filament.

The aims of this study is characterizing morphological forms and DNA barcode based on the 16S rRNA gene of *Arthrospira* (*Spirulina*) cultured in fertilizer and waste water effluent of *Clarias* pond farming media, and determining optimal pH value and salinity of culture media for growth and phycocyanin production, especially in *Clarias* pond farming waste water media and morphological changes of their filament.

### MATERIALS AND METHODS

### Arthrospira cultured in agar media

Bacto agar was weighed as much as 2 g dissolved in 100 ml of water. The water used was swamp water and catfish culture waste that has been filtered and sterilized using an autoclave. Sterilized swamp water was then added with 0.02 gram MgSO4 fertilizer; CaCl2 0.004 gram; EDTA 0.008 gram; urea 0.03 gram; ZA (Sulphate of Ammonia) 0.132 grams; 0.4 gram baking soda; AB solution 1 ml mix A solution (Calcium Nitrate 64,26%, Potassium Nitrate 33,66%, Fe EDTA 2,08%) 2 grams / 10 ml and B solution(Potassium dihydrophosphate 25,83%, Ammonium sulfate 9,41%, Potassium sulfate 2,78%, Magnesium sulfate 60,91%, Cupric sulfate 0,03%, Zinc sulfate 0,12%, Boric acid 0,31%, Manganese sulfate 0,62%, Ammonium heptamolybdate 0.01%) 2 grams/10 ml water and TSP (Triple Super Phosphate) 0.05 grams were then homogeneous using magnetic stirrers. Next, wasted water was sterilized by an autoclave then cooled. Bactoagar was added to the technical fertilizer and waste solution to be homogenized using a magnetic stirrer and then boiled using a hot plate until all the ingredients dissolve and then autoclave again. The agar media was made with a pH of 7 and a salinity of 10 ppt or 10 g.L-1 (Hidayani et al, 2019).

Arthrospira cultivated in liquid media was taken 100 μl using micropipette and spread to the surface of a petri dish containing bactoagar media by using a sterilized spreader rod. Petri dishes were wrapped in wrapping plastic and then given a lamp lighting (light intensity 2000-4000 lux) with a dark: light ratio = 0:24 hours. Arthrospira was observed every day until it grows blue green. After growing, Arthrospira was re-cultured in agar media by the 4 quadrant scratch method. The cultures were used as isolate samples for determining DNA barcodes. The biomass of Arthrospira was isolated from commercial Spirulina TopSpira East Jakarta, Indonesia.

The commercial Arthrospira was cultured in technical fertilizer media directly, and we used the media based on our previous study about fertilizer media in laboratory scale (Laboratorium of Aquaculture, Faculty of Agriculture, Sriwijaya University). The result showed that this fertilizer media can substitute Zarrouk Medium (ZM) for growing Spirulina biomass in cheaper medium than ZM (Wijayanti et al, 2018).

### **DNA** extraction

DNA extraction was carried out according to procedures in which there was a Presto TM Mini gDNA Bacteria Kit (Geneaid Biotech Ltd.). DNA extraction consisted of several stages: sample preparation, lysis, purification, and precipitation or washing. The sample used was 0.15 grams of wet weight for one extraction (Geneaid manual).

### **DNA** amplification

The process of DNA amplification using the PCR (Polymerase Chain Reaction) method was performed using 2 µl forward primers 63f (5'-CAGGCC TAA CAC ATG CAA GTC-3 ') and reverse primer 1387r (5'-GGG CGG WGT GTA CAA GGC-3 ') (Marchesi et al., 1998). The total composition of the PCR mixture was 50 µl which consisted of 25 µl Go Taq Green, 13 µl NFW (Nuclease Free Water) and 8 µl Arthrospira DNA extraction template. DNA amplification was carried out in stages: the initiation cycle at 95°C for 5 minutes, followed by 30 denaturation cycles at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, then the extension stage at 72 °C for 1 minute, and the final stage 72 °C for 7 minutes (Lee et al., 2003).

### Electrophoresis

Electrophoresis was carried out using 1% agarose gel at 75 V for 35 minutes. Agarose that have been electrophoretic was immersed with a mixture of 10 µl diamond dye solution and 100 ml TAE 1x buffer solution for 30 minutes without exposure to light. The results were visualized through gel documentation by observing DNA migration using a transiluminator UV.

### Gene Sequencing

Arthrospira DNA samples that were successfully amplified using PCR were then sequenced in the fragments of 16S rRNA gene. The amplified products were sequenced through the services of the Macrogen Institute in Jakarta. The DNA sequences obtained in the form of fasta format were aligned using MEGA 6.0 software and then uploaded through the Basic Local Alignment Search Tool (BLAST) program. BLAST was a program to search for and analyze the homology of an organism's sequence, on the ncbi.nlm.nih.gov website so that its homology can be identified with other genus Arthrospira 16S rRNA gene sequences registered in the GenBank database. The genetic distance and phylogenetic trees between genera were constructed using the Neighbor Joining (NJ) method. The phylogenetic tree was constructed through the Mega 6.0 software application using the Neighbor-Joining (NJ) method of the Maximum Composite Likelihood model and Substitutions to include d: Transitions + Transversions with 1000x boostrap. Meanwhile Arthrospira morphologicals form analysis were presented in the form of images and discussed descriptively by referring to the appropriate literature.

### Optimization of pH and salinity for growing Arthrospira in Catfish farming wasted water

The experimental design for optimizing pH and salinity media for growing Arthrospira in Catfish farming wasted water was a Factorial Completely Randomized Design (CRD) consisting of the first factor with 3 treatments and the second factor with 4 treatments and 3 replications. The first factor was the difference of pH in culture media, including P1: culture media pH 6.5  $\pm$  0.2, P2: culture media pH 8.5  $\pm$  0.2 and P3: culture media pH 10.5  $\pm$  0.2. The second factor was the difference of salinity in culture media i.e. S1: salinity of 0 ‰, S2: salinity of 10 ‰, S3: salinity of 20 ‰, and S4: salinity of 30 ‰.

### **Culture preparation**

The equipment used in this study was sterilized using 70% alcohol to minimize the contaminants that inhibit the productivity of Arthrospira. The containers used plastic bottles with capacity of 5 L volume of 36 units. The plastic bottle were sterilized using a potassium permanganate solution (2 mg. L-1). Catfish culture wastewater obtained from catfish farming ponds measuring 2 m x 1 m x 1 m, and high of water media was 20 cm (Figure 1). The density used in the pond was 330 fish.400 L-1 with 150 grams fish-1, maintained for 2 months by providing artificial feed (protein 31%-33%), twice per day at satiation. Catfish culture wastewater was previously sterilized by boiling in an autoclave and then cooled, while the steril wastewater was treated with salinity. In treatments S1, S2, S3 and S4 were added with salt until salinity was obtained according to the treatment. The wastewater media had a pH of 7.3, therefore there was an addition of HCI I N of 0.75 ml L-1 in P1 treatment to reach a pH of 6.5. Meanwhile, in treatments P2 and P3, to get a pH of 8.5 and pH 10.5 there was an addition of NaOH 8 N as much as 0.07 ml L-1 and 0.45 ml L-1.



Figure 1. Catfish farming pond



Figure 2. Arthrospira cultivation

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### Arthrospira cultivation.

Arthrospira previously used was cultured in catfish culture wastewater for culture stock with a density of 2 g L<sup>-1</sup>. The stock was taken as much as 400 ml in 3600 ml of catfish culture wastewater in accordance with treatment. Aeration was used for agitation, the lighting using 36 watt TL lamps for 24 hours day<sup>-1</sup> during maintenance (Figure 2). Harvest of biomass. The components of wastewater were total phosphorus 2,6 mg.L<sup>-1</sup>, total nitrogen 1,9 mg.L<sup>-1</sup>, total organic carbon 11,4 mg.L<sup>-1</sup>.

Harvest of the biomass was after exponential phase by filtering. The biomass was dried using an oven for 14 hours at 40 °C (Hidayani et al., 2019). The dry biomass was used for the phycocyanin extraction process.

### Phycocyanin extraction

The dry biomass was 0.04 g added by 1 ml of phosphate buffer pH 7, then homogenized and frozen in the freezer for 24 hours at a temperature of -4 °C. After 24 hours from the freezer, thawing process for 15 minutes. Samples were centrifuged for 30 minutes at 3000 rpm. After that, the sediment and the supernatant were separated. The resulting supernatant was phycocyanin which be analyzed using the Bennett and Bogorad method (1973).

### The density of Arthrospira biomass.

Biomass density measurements were performed at each treatment and 3 replications every day at the same hour. The density of biomass was 1 ml of sample in each treatment with 3 replications. The 1 ml of sample into aluminum bowl. The sample and the aluminum bowl were weighed, then dried in the oven for 14 hours at 40 °C. The sample of water that had dried was weighed again. The dry biomass weight of *Arthrospira* biomass was converted to g L<sup>-1</sup>.

The growth rate of *Arthrospira* can be calculated using the following formula according to Vonshak (1997):

$$\mu = \frac{\ln Nt - \ln N_0}{t} \ge 100\%$$

Note:  $\mu = \text{daily growth rate (% days^{-1})}$   $t = \text{time (days) from N_0 to N_1}$  $N_0 = \text{initial density (g L^{-1})}$ 

 $N_t$  = density at the time t (g L<sup>-1</sup>)

Measurement of total nitrogen and phosphate content in culture media was carried out at the beginning and the first day after the peak phase of each treatment..

The measurement of phcocyanin refers to Bennett and Bogorad (1973). The absorbed supernatant was measured using a spectrophotometer at wavelengths of 615 nm and 652 nm.

Rendement of phycocyanin (mg g<sup>1</sup>) = <u>C-phycocyanin x V</u> DB ISSN: 1412-033X E-ISSN: 2085-4722 DOI: 10.13057/biodiv/d2112xx

Rendement percentage of phycocyanin (%) = Rendement of phycocyanin (mg g<sup>-1</sup>) x 100%

Note: C-phycocyanin = C-phycocyanin consentration (mg.  $mL^{-1}$ )

V = Solvent Volume (ml)

DB = Dry Biomass (0.04 g)

0.474 and 5.34 = coefficient of extinction (Bennett and Bogorad, 1973)

The results were submitted to simple analysis of variance tests (ANOVA) (p < 0.05) and in the case of significant differences, the means were compared by the Least Significant Differences test (p<0.05).

### RESULTS AND DISCUSSION

### Morphology of Arthrospira

Arthrospira was cultivated using two different fertilizer media namely technical fertilizer and waste media. The morphology of commercial Arthrospira before fertilizer treatment was presented in Figure 4.

The results of the identification of isolates showed that the isolate had a twisted filament shape resembling a spiral (helical). Based on Davis's identification book (1955), it is known that the isolate used in the study was *Spirulina* (*Arthrospira*) platensis. *Arthrospira* is cyanobacteria belonging to the order Oscillatoriales which has a filament (trichome) that resembles a spiral (helical) but does not have heterocyst cells (Sze, 1998). Heterocyst cells are special thick-walled cells that play a role in nitrogen fixation from the air (Issa et al, 2014). In this study *Arthrospira* cultured in different media had several linear/straight morphologies.

Based on Figure 2, Arthrospira which was cultured on technical fertilizer media has a longer and spiraling morphological form compared to another cultured on waste media. Their filaments have more linear morphological form, some spirals but not too long. According to Astiani et al (2016), Arthrospira growth is influenced by nutritional and environmental factors. Wang and Zhao (2005) explained that linearization that occurs in Arthrospira is a variation on the genetic level that can be caused by environmental factors such as lack of nutrition and high light intensity. In this study, isolates were cultured with the same light intensity of 2000-3000 lux with a light dark ratio of 0:24 hours. Linear filaments in AW have a lower metabolic rate compared to helical filaments. This is one of the adaptive mechanisms for Arthrospira to survive some environmental conditions that are not appropriate.

Yadav et al (2020) showed that helical and linier morphotypes of Arthrospira sp. display genomic differences. Vonshak (2000) showed that polyphasic in Arthrospira morphotypes can caused by: growing in agar/solid media, light stress-photoinhibiton, irradiation, and temperature, effect of physical and chemical conditions. Arthrospira is prokaryotic organism, so it is easy to mutation and change sequences in their genome. BIODIVERSITAS Volume 21, Number 12, December 2020 Pages: xxxx ISSN: 1412-033X E-ISSN: 2085-4722 DOI: 10.13057/biodiv/d2112xx



Figure 3. Dry biomass of Arthrospira platensis after oven



Figure 4. Morphological identification results of Arthrospira isolate (CA "Commercial Arthrospira"; AF= commercial Arthrospira cultured with technical fertilizer media; AW= commercial Arthrospira cultured with waste media) 40x magnification.

Tabel. 1. The results of the BLASTn analysis of Arthrospira samples cultivated in technical fertilizer and waste medium with data in Genbank

Description	Identity (%)	Access code	Sample origin
Arthrospira (fertilizer media) (AF)			
Arthrospira platensis petH	100	AB113346	Japan
Spirulina platensis CCC 478	90,48	JX014313.1	India
Spirulina platensis cyaG	94,4	D49531.1	Japan
Arthrospira platensis PCC 7345	90,12	JN831264.1	USA
Arthrospira maxima EEW2	74,4	HQ008225	Australia
Arthrospira (waste media) (AW)		545.2075.11106/0 AC	
Arthrospira platensis petH	94,3	D49531.1	Japan
Arthrospira platensis DKCAS2	81,4	MG912588.1	India
Spirulina platensis CCC 478	74,4	JX014313.1	India
Arthrospira maxima str. Lefevre 1963/M-132-1	73,3	FJ798612	Venezuella
Arthrospira maxima EEW2	72,2	HQ008225	Australia

### **Phylogenetic Tree**

The results of the 16S rRNA encoding gene sequences from AF and AW isolates were traced to other *Arthrospira* isolates present in GenBank through the BLAST program. The results of the BLASTn analysis of *Arthrospira* samples cultivated in technical fertilizer and waste media with data in Genbank are presented in Table 1. Table 1 results of the BLAST analysis show the closeness between AF and AW isolates with other isolates in GenBank. It show that *Arthrospira* technical fertilizer isolates and *Arthrospira* waste isolates have the closest homology to *Arthrospira platensis* petH species from Japan with percentage values respectively 100% and 94.3%. Genetic distance was used to see kinship relationships from Arthrospira both AF and AW samples with sequential data from Genbank. AF isolate indicated a genetic distance of 0.068 with AW isolates. AW and AF isolates showed the lowest genetic distance respectively 0.089 and 0.060 with Arthrospira platensis petH from Japan. Analysis based on genetic distance showed that both isolates were belong to the same species namely Spirulina platensis, however the genetic distance was 0.068 (6.8%) meaning that there are intraspecies variations in the sample caused by mutations.

Phylogenetic tree Arthrospira isolates from technical fertilizer and waste media were presented in Figure 5. The phylogenetic tree is a two-dimensional graph showing relationships between organisms or population classifications based on their evolutionary history. The result of phylogenetic tree construction showed that both samples formed branches with a cluster. Phylogenetic tree from AF and AW isolate sequences formed cluster was separate with several other *Arthrospira* species from GenBank data.

The AF isolates had a close relationship with *Arthrospira platensis* petH species from Japan with a bootstrap value of 95%. Hadiati (2003) states that bootstrap analysis is performed to determine the level of confidence in grouping. Bootstrap value is considered high because according to Hall (2001), a clade can be trusted with a bootsratp value of 90%. In addition, Hillis and Bull (1993) state that bootstrap analysis with values of 70% or higher indicate a reliable grouping. The AW isolates formed a separate brach of AF isolates and *Arthrospira platensis* petH species. Genetically, they had diverse, and adapted to environmental conditions. The AW isolate indicated different strain from AF isolate groups. Ballot et al. (2004) stated that *Spirulina* from the same species and cultured under different conditions can form a separate subcluster with a bootstrap value of 79%. Zhao et al. (2006) identified and analyzed the number of restrictionmodification genes in the cyanobacterial genome, seeing that more restriction-modification genes were found in cyanobacterial filaments (*Anabaena, Spirulina* and *Nostoc*) than dispenses (*Synechocystis, Synechococcus* and *Prochlorococcus*) this was due to the organism adapting to various environmental conditions, or the many variations in sources of nutrients that cause mutations.

### Density and Growth Rate of Arthrospira cultured in wastewater of catfish farming

The biomass of Arthrospira displayed mechanism of adaptation in culture media. The wastewater media could make different charateristic of growth as like as filament. The maximum densities of Arthrospira cultured in wasted water were achieved on a different day. The daily density of Arthrospira during culture can be seen in Figure 6.



Figure 5. Phylogenetic analysis with 1000 bootstrap AW (Arthrospira cultured in waste water media) and AF (Arthrospira cultured in fertilizer media)



Figure 6. Cell density (dry weight with a moisture content of 1.2%) of Arthrospira in catfish rearing pond waste water

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The graph presented in Figure 6, it show that in the culture period from day 1 to day 2, Arthrospira in each treatment experienced slow growth, because the cells were still adapting to their new environment. The exponential phase for the treatment of P2S2, P2S3, and P3S3 last from day 1 to day 8 of the culture period. In the treatment of P2S1, P2S4, P3S1 and P3S2 take place from 2nd until 8th day of the culture period. While the treatment of P1S1, P1S2, P1S3, P1S4, and P3S4 lasted from 3rd - 8th day of the culture period. The decreasing Arthrospira density for the treatment occurred from the day 9 to the day 11 of the culture. Lesmana et al. (2019) explained that the adaptation phase lasts from day 0 to day 1, while the exponential phase occurs from day 1 to day 7, and experiences a stationary phase from day 7 to day 9 then enters the death phase after the 7th day and 9th day. The decrease of density could because of reducing the nutrients in the culture media. Soni et al. (2019), the concentration of nutrients in the media decreased after reaching the peak period. This is due to the higher density of Arthrospira in the culture media.

The maximum density of *Arthrospira* could be achieved on different day, between 5 - 8 days after culturing. The mean of maximum density could be 0.433-0.867 g L<sup>-1</sup> of dry biomass which cultured in catfish rearing waste water. The maximum cellular density of *A. platensis* which cultured in nile fish rearing waste water, resulted in the production of 0.22 g L<sup>-1</sup> of dry biomass and maximum productivity of 0.03 g L<sup>-1</sup> day<sup>-1</sup> (Nogueira et al, 2018). The catfish rearing pond waste water has high potential as cultivation media for Arthrospira production.

The analysis of variance showed that differences in pH, salinity and interaction between factors (pH and salinity) ISSN: 1412-033X E-ISSN: 2085-4722 DOI: 10.13057/biodiv/d2112xx

significantly affect the maximum density and growth rate of *Arthrospira platensis*. The results of the LSD  $_{0.05}$ maximum density test and growth rate sequentially were presented in Table 1 and Table 2. LSD  $_{0.05}$  test results on the main factors of differences in pH, density and growth rate of *Arthrospira platensis* in the P2 treatment (pH 8.5 ± 0.2) were significantly higher than those in the P1 (pH 6.5 ± 0.2) and P3 treatments (pH 10.5 ± 0.2). According to Ismaiel (2016), the highest biomass of *Arthrospira platensis* is produced in media with a pH of 8.5-9.5. Although *Arthrospira platensis* can tolerate a wide pH range, a pH range farther from its optimal pH can reduce its growth rate. A low growth rate will also cause low biomass production.

Furthermore, the different salinity treatment factors showed that the maximum density and growth rate in treatment S3 (salinity of 20 ‰) were significantly higher compared to S1 (salinity of 0 ‰), S2 (salinity of 10 ‰) and S4 (salinity of 30 ‰) treatments. The S1 and S4 treatments were not significantly different and were the treatments that produced the lowest density compared to other treatments. Table 1 and Table 2 showed that the highest density and daily growth rate was found in the S3 (salinity of 20 ‰) treatment. This is supported by the results of Kouhgardi et al. (2015), that Arthrospira platensis cultured on Conway media was able to produce the highest density of 912.07 mL-1 cells at a salinity of 20 ‰. While the density and growth rate between S1 and S4 treatments showed no significant difference. This is because the salinity of 0-30 % is still within the range of salinity that can be tolerated by Arthrospira. Ughy et al. (2015) said that Arthrospira platensis is one of the species of Cyanobacteria that can grow in an euryhaline environment.

Single Influence of pH (P)		Single Influe (LSD)	Main influence of pH (P)		
	S1(0 %)	S2 (10 %)	S3 (20 ‰)	S4 (30 %)	(LSD 0.05=0.055)
P1 (pH 6.5)	0.433 a	0.633 b	0.767 cdef	0.467 *	0.575 ª
P2 (pH 8.5)	0.767 cdef	0.833 ef	0.867 f	0.800 def	0.817 °
P3 (pH 10.5)	0.667 bc	0.733 bode	0.767 cdef	0.700 bcd	0.717 <sup>b</sup>
Main influence of Salinity (S) (LSD 0.05=0.062)	0.622 ª	0.733 <sup>b</sup>	0.800 °	0.656 <sup>a</sup>	

Table 1. Maximum density of Arthrospira platensis (g L-1)

Table 2. The growth rate of Arthrospira platensis cultured in pH and salinity treatment(% day-1)

Single Influence of pH (P)		Single Influ (LSI	Main influence of pH (P)		
· · · · · · · · · · · · · · · · · · ·	S1(0 ‰)	S2 (10 ‰)	S3 (20 ‰)	S4 (30 ‰)	(LSD 0.05-1.012)
P1 (pH 6.5)	14.333 <sup>a</sup>	18.659 cd	19.192 cde	13,348 ª	16.383 <sup>a</sup>
P2 (pH 8.5)	18.343 <sup>bod</sup>	20.570 de	22.026 e	20.723 de	20.416 b
P3 (pH 10.5)	20.023 de	19.527 <sup>cde</sup>	20.623 de	16.417 abc	19.147 <sup>b</sup>
Main influence of Salinity (S) (LSD 0.05=1.861)	17.566 <sup>a</sup>	19.585 <sup>b</sup>	20.614 <sup>b</sup>	16.829 ª	In Concernant I
Mater					

Note

%/oo mean part per thousand (ppt), it is not percent (%). Salinity of fresh water (0 ppt), brackish water (5-20 ppt) and sea water (more than 25 ppt). The salinity used in this study still supports the growth of Arthrospira (Vonshak, 1997).

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The interaction between pH and salinity factors showed that the density and growth rate in treatment P2S3 (pH 8.5 ± 0.2 and salinity of 20 ‰) were significantly higher compared to other treatments. At maximum density, treatment P2S3 was not significantly different from treatments such as P1S3, P2S1, P2S2, P2S4, and P3S3. While in the growth rate, treatment P2S3 was not significantly different from P1S3, P2S2, P2S4, P3S2 and treatments P3S3. Table 1 and Table 2 show that treatment P2 (pH  $8.5 \pm 0.2$ ) is more dominant causing higher density and growth rate of Arthrospira platensis despite being in different salinity ranges. As for the treatments P1 (pH 6.5 ± 0.2) and P3 (pH 10.5 ± 0.2) provide the highest density when combined by the treatment S3 (salinity 20 ppt). The treatment of P1, P2, and P3 (pH 6.5 ± 0.2, 8.5 ± 0.2, and 10, 5 ± 0.2) and S1, S2, S3 and S4 treatments (salinity of 0 36, 10 36, 20 36, and 30 36) still support the growth of Arthrospira platensis with the best treatment found in the combined treatment P2S3 (pH 8.5 ± 0.2 with salinity of 20 % 3.

Mismatch of pH will cause lysis and can change the shape of pigment growth (Hariyati, 2008). The process of photosynthesis affects the pH value. In daylight, aquatic plants release carbon dioxide from water for use in photosynthesis. The release of carbon dioxide by plants occurs through respiration. When carbon dioxide is released, carbonate builds up and hydrolyzed so that the pH of the water will increase (Boyd, 1990). Prasadi (2018) showed that growth of Arthrospira could be inhibited if it was in the pH range above 10.5 or less than 7. Salinity is one of the factors that can influence osmotic pressure for Arthrospira as like as others of microalgae. Pisal and Lele microalgae can experience cell shrinkage in (2005)conditions outside the cell salinity higher than inside the cell (hypertonic), and vice versa under conditions of low salinity outside the cell (hypotonic) cell swelling will occur due to water molecules outside moves into the cell. This condition affects the process of photosynthesis, and makes microalgae to produce secondary metabolites in the form of β-carotene to sustain life against changes in salinity in culture media. While in isotonic conditions, cell fluid is isotonic to its external media which causes low active ion transport and osmoefector exchange, making the Na-K-ATPase enzyme activity at a maximum level and more energy will be utilized for growth (Rahmawati et al., 2012 ). The optimal combination of pH and salinity causes the growth of Arthrospira to be maximal. The optimal salinity range for Arthrospira is between 15-20 ‰, from the related research showed that the results of Arthrospira culture with 20 ‰ of media salinity, pH 7.5-8.5 using fertilizer media (0.010 g L-1 TSP, 0.030 g L-1 Urea, and 0.030 g L-1 ZA) and a culture periode of 9 days produced a dry weight of Arthrospira of 0.0375 g L-1 (Prasadi, 2018).

### Rendement of phycocyanin

The rendement of Arthrospira was presented in Table 3. The pH condition of maintenance media can be affect of protein content in Arthrospira cells. The results of LSD 0.05 on the main factor of pH showed that the rendement of phycocyanin Arthrospira in treatment P2 was significantly ISSN: 1412-033X E-ISSN: 2085-4722 DOI: 10.13057/biodiv/d2112xx

higher compared to other treatments. Taufiqurrahmi et al. (2017), the amount of *Arthrospira* biomass influences the high content of phycocyanin. Table1 showed that the highest of *Arthrospira* biomass was found in treatment P2. It showed that the highest *Arthrospira* biomass produced the highest rendement of phycocyanin (Table 3). The culture medium of *Arthrospira* pH of 8.5 produced the highest C-phycocyanin content (Ismaiel et al., 2016). Rahmawati et al. (2017) said that the higher of C-phycocyanin followed the higher of rendement of phycocyanin.

LSD 0.05 showed that the main factor of salinity showed the rendement of phycocyanin in treatment S3 (salinity of 20 ‰) was significantly higher than other treatments. It is thought that the difference in salinity treatment has an impact on the external osmotic pressure of Arthrospira cells which results in changes in cell composition especially phycocyanin. Sodium will flow into the cell and cause the release of phycobilin (phycoeritrin, phycocyanin and allophycocyanin) from PS II (Photosystem II) and stop the electrons transporting to PS I (Photosystem I) followed by activation of the protective mechanism. Arthrospira will produce carbohydrates to balance intracellular osmotic pressure and require more energy to remove sodium ions from cells. In this case it will produce ammonium assimilation causes inhibition of protein synthesis (Zhou et al., 2017).

The environment includes the availability of nutrients, pH, salinity, light and temperature can affect the growth and accumulation of biopigments from microalgae (Sharma and Tiwari, 2014). The condition of culture media is able to influence the growth phase of Arthrospira, causing changes in the composition and proportion of phycobilin (phycoeritrin, phycocyanin and allophycocyanin) (Simeunovic et al., 2013). The results of the LSD 0.05 was showed that the rendement of phycocyanin Arthrospira on the interaction between factors in treatment P2S3 was significantly higher than in other treatments, but it was not significantly different from treatment P2S2. This showed from the density and growth rate of Arthrospira. The increasing salinity will cause maintenance media to be hypertonic towards cells and result in poor absorption of nutrients by cells. These cells could reduce in protein and increase in carbohydrates from Arthrospira cells (Ravelonandro et al.,2011).

Production of phycocyanin was able to reach 12.4 % -17.6% of biomass dry weight of Arthrospira cultured in Zarrouk Medium (ZM) (Prates et al., 2018; Garcia-Lopez et al., 2020). There are several factors that affect the rendement of phycocyanin include temperature, extraction time, mixing rate, biomass, type of solvent and the ratio of biomass to the solvent (Taufiqurrahmi et al., 2016). The content of phycocyanin in cyanobacteria increases when grown in low light intensity. Phycocyanin is a pigment associated with protein, polar and water soluble. The protein content of microalgae are influenced by environmental conditions such as temperature, age of culture, light intensity, pH, salinity, and nutrient limits (especially nitrogen). Nitrogen is an essential element needed for the synthesis of accessory pigments and chlorophyll. When microalgae are growing fast, it requires large amounts of nitrogen and could consume phycocyanin as an alternative source of nitrogen for the production of biomass (Hsieh-Lo et al., 2019). It must be optimized for biomass production and phycocianin content. The higher concentration of phycocyanin will be followed by the rendement of phycocyanin. In this study, the phycocyanin content is lower than previous study, because of different media for culturing Arthrospira. The nutrient from ZM (pro analysis substances) is more complete for growing and forming phycocyanin than waste water catfish pond media, especially the trace mineral in ZM.

### **Reduction of Total Nitrogen and Phosphate Content**

The percentage reduction of total nitrogen in the culture media was presented in Table 4.

Table 3. Phycocyanin (%)	yield in Arthrospira dry	biomass at 8 days after inoculation

Single Influence of pH (P)		Single Infl (LS	Main influence of pH (P)		
	S1	S2	\$3	S4	(LSD 0.05=0.096)
Pl	7.881 <sup>a</sup>	8.783 °	9.441 <sup>d</sup>	8.387 b	8.623 <sup>a</sup>
P2	9.657 °	10.906 h	11.347 <sup>i</sup>	10.423 8	10.583 °
P3	8.970 °	9.408 d	10.134 f	9.262 d	9.444 <sup>b</sup>
Main influence of Salinity (S) (LSD 0.05=0.111)	8.836 ª	9.699 °	10.307 <sup>d</sup>	9.357 b	

Table 4. Reduction of total nitrogen content in Arthrospira culture medium (%)

Single Influence of pH (P)		Single In (L	Main influence of pH (P)		
	S1	S2	\$3	S4	(LSD 0.05=0.645)
P1	80.990 ª	82.250 ab	83.767 cde	81.897 ab	82.226 ª
P2	83.880 de	84.377 ef	85.420 f	84.857 ef	84.633 °
P3	82.940 <sup>bcd</sup>	81.143 ª	84.950 ef	84.813 ef	83.462 <sup>b</sup>
Main influence of Salinity (S) (LSD nos=0.745)	82.590 ª	83.856 <sup>b</sup>	84.712 °	82.603 °	

The mechanism for removing nitrogen in water is determined by several factors, including bacterial activity (Gersberg et al., 1986), uptake by plants (Breen, 1990) and evaporation (Sanchez-Monedero et al., 2001). In Cyanobacteria, nitrogen is a macronutrient that plays an important role in the formation of biochemical compounds such as nucleic acids (DNA, RNA), amino acids (protein) and pigments (chlorophyll and phycocyanin) (Markou et al., 2014). The results of the LSD 0.05 on the main pH factor showed that the reduction in total nitrogen content in Arthrospira culture media in treatment P2 was significantly higher than in treatments P1 and P3. This is due to the higher density of Arthrospira in treatment P2 compared to other treatments, so that the utilization of nitrogen by Arthrospira in culture media is greater than others. Markou et al. (2014) showed that the higher density of Arthrospira could be higher the absorption of nutrients including nitrogen.

The value of reducing total nitrogen content in S3 treatment was significantly higher than for other treatments. This showed that treatment S3 caused *Arthrospira* to absorb nitrogen higher than other treatments in line with the high density and rendement of phycocyanin *Arthrospira* obtained in this study. Jabeen and Ahmad (2011) showed that salinity in culture media influences nitrogen absorption and protein biosynthesis. Reduction of total nitrogen content in *Arthrospira* culture media due to interactions between pH and salinity factors showed that treatment P2S3 was significantly higher than other treatments. With the optimal conditions (pH and salinity),

Arthrospira is able to make maximum use of nitrogen. This can be seen from the highest density, growth rate and rendement of phycocyanin found in treatment P2S3. The amount of nitrate and phosphate decreases with increasing growth of *Chlorella vulgaris* and *Arthrospira platensis* (Sayadi et al., 2016). This is because algae have the ability to absorb nutrients such as nitrogen and phosphate are use to carry out photosynthesis and protein production. Reduction of phosphorus content in waters is influenced by the process of absorption, complexation, deposition and assimilation (between microbes and plant biomass) (Tanner et al., 1999).

The results of LSD  $_{0.05}$  on the main factor of pH showed that each treatment did not significantly affect the reduction of phosphate content in *Arthrospira* culture medium. Plants can only absorb phosphorus in the form of H<sub>2</sub>PO<sub>4</sub> and HPO<sub>4</sub>-<sup>2</sup> free orthophosphate ions (Becquer et al., 2014). The orthophosphate content decreases with increasing media pH. Cerozi and Fitzsimmons (2016) showed the orthophosphate content increases in the pH range from 5.5 to 8.5 and decreases when pH 10. The fall in the orthophosphate content at pH 10 is due to an increase in calcium phosphate formation. The value of reducing the phosphate content in *Arthrospira* culture medium was presented in Table 5.

The salinity factor, administration of different salinity in each treatment has no significant effect on reducing the phosphate content. Bassin et al. (2011) explained the reduction of phosphorus will be inhibited when a combination of Cl<sup>-</sup> and nitrite and Cl<sup>-</sup>concentration more

than 2.5 g L-1. The interaction effect of differences in pH and salinity, each treatment had no significant effect on reducing phosphate content. Hua-Sheng et al. (1995) showed that the utilization of Dissolved Organic Phosphorus (DOP) can be through active uptake into cells or by extracellular mineralization by phosphatase enzymes. However, most DOP compounds cannot be assimilated directly with microalgae because they have been mineralized. Markou et al. (2014) stated that phosphorus is a macronutrient that plays an important role in the preparation of nucleic acids (RNA and DNA), phospholipids and energy-carrying molecules (ATP). The phosphorus content in plants is lower than Ca, N, and K (Sasaqi et al., 2018). Although the analysis of variance shows that the results are not significantly different, but the highest phosphate reduction still existed in treatment P2S3.

### Morphology of Arthrospira in various pH and salinity media

Morphology of Arthrospira was affected by increasing or decreasing physical or chemical factors in their culture media. Salinity and acidity value combination at this study didn't change the filament of Arthrospira significantly as their filament of Arthrospira under microscope with 100x magnification (Figure 7). The morphological form of Arthrospira were not different in all of pH and salinity treatments. The waste water catfish pond media could affected in the linierizing filament. This study indicated that salinity and acidity of culture media didn't effect on various form filament, either straight and helical.

Table 5. Reduction of phosphate content in Arthrospira culture medium (%)

Single Influence of pH (P) -		Single Influer	Mala la Guarda de Call (Ba		
	S1	S2	\$3	<b>S4</b>	<ul> <li>Main influence of pH (P)</li> </ul>
P1	70.500	71.667	73.000	72.333	71.875
P2	74.667	74.000	74.667	72.667	74.000
P3	70.333	70.333	73.667	72.667	71.750
Main influence of Salinity	71.833	72.000	73.778	72.556	



Figure 7. Morphology of Arthrospira in catfish wastewater culture media at several treatment of pH and salinity

The straight filaments were observed for Arthrospira strains during two years of cultivation, and their presence in Arthrospira sp. Nigrita C1 cultures was constant. The various morphological plasticity, greatly influenced by the growth stage and exogenous parameters, such as temperature and light intensity, was observed for Arthrospira strains (Papapanagiotou and Gkelis, 2019). There are indications that adaptability to change in environmental conditions is relatively rapid and also subsequent changes at the genetic level can be realized quickly. This means that we can easily find different genotypes in various stable, ecologically different habitats. Morphologically similar strains were cultured for a long time under uniform and stable conditions (Komarek, 2016). But the morphological changes couldnot be effected by acidity and salinity of culture media.

### Conclusions

Arthrospira that is cultured on waste media (observed in liquid culture) indicated some short and linear filaments. Identified Arthrospira had a genetic distance of 6.8% between AF and AW isolates. AF isolates had a close relationship with Arthrospira platensis petH species originating from Japan (bootstrap value of 95%) while AW isolates form phylogenetic branches which are separated from AF isolates and Arthrospira platensis petH species originating from Japan (bootstrap value 85%). The catfish culture wastewater media at different pH and salinity affects the density, growth rate and rendement of phycocyanin Arthrospira platensis. The highest density, growth rate and rendement of phycocyanin was in P2S3 treatment (pH 8.5 ± 0.2 and salinity of 20 ‰) which produced a maximum density of 0.867 g L1, growth rate of 22.026% day-1 and the rendement of phycocyanin of 11.334 %.

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No	<b>Reviewer's Questions</b>	Author's Answer
	From Reviewer C	
1	There is no control+/- to compare the results of growth, phycocyanin and identity blasts. The authors need to bring the results of CA (commercial Arthorospira) and AF to see the differences between the treatments with Control. The comparison only in morphology has been brought. They should bring this comparison in the other factors they worked like growth, phycocyanin, N and P reduction etc.	We didn't give control+/- to compare the result of growth, phycocyanin and identity blast in our study, because we didn't have the commercial Arthrospira cultivation. We grew the commercial Arthrospira (in commercial name 'Spirulina') in fertilizer media. The picture of CA morphology was taken at the first time commercial isolates arrived. The picture of AF morphology was taken after cultivation of the CA isolates in fertilizer media, and the AW morphology picture was taken after cultivation of AF isolates in waste water media. To compare the result of growth, phycocyanin, identity blast, N and P reduction, we used data from
2	Still it's not clear how the pH and salinity along with some other factors in the waste water can change genome of an organism during a week.	<ul> <li>We tried to cultivate the biomass of Arthrospira in fertilizer media for scaling up, and the biomass of Arthrospira from fertilizer media was continued to cultivating in waste water media. The cultivation of Arthrospira needed several week for scaling up. We used fertilizer medium for cultivation for 2 weeks, isolated for purification in agar media for 3 weeks, and transferring in liquid media (fertilizer media) and growing them in 50 ml volume Erlenmeyer for 8 days, and then they had been scaling up to 1 liter. The stabile cultivation of Arthrospira (fertilizer media) was transferred in waste media cultivation. Production time of Arthrospira in same scale of cultivation was 8 days. The Arthrospira cultured in waste medium was isolated to agar media enriched with waste water from catfish pond.</li> <li>We didn't know that the waste water can change genome of Arthrospira. We extracted the DNA of AF and AW from agar media. The DNA genomes were amplified by the 16SrRNA primer (Marchesi, 2003). The amplicons were sequenced for analyzing their genetic drift</li> </ul>

# Table 1. The author's answers to the reviewers' questions

		for different cultivation media. The different of morphotypes between AF and AW can be caused polyphasic based on physiology of adaptation to heterotrophic media (waste media) only or changing their genomes. The data showed that it was genetic drift because of their different sequences after aligned with data from gene bank. Yadav et al (2020) showed that helical and linier morphotypes of Arthrospira sp. display genomic differences. Vonshak (2000) showed that polyphasic in Arthrospira morphotypes can caused by: growing in agar/solid media, light stress- photoinhibiton, irradiation, and temperature, effect of physical and chemical conditions. Once a strain has converted to the straight form, either naturally or due to physical or chemical treatments, it does not usually revert to the helical form. This may be due to a mutation affecting some trichomes under certain growth conditions. The common occurrence of straight trichomes in cultures of Arthrospira also suggests that the helical character may be carried on plasmids, but no one has yet demonstrated the existence of plasmids in Arthrospira or Spirulina strains. Arthrospira is
		mutation and change sequences in their
	From Reviewer V.	
1.	What the source of <i>Arthrospira</i> ?	It was from commercial Spirulina "TopSpiraSpirulina" East Jakarta
2	Why not use a specific Zarrouk medium?	Because the commercial Spirulina was cultured in technical fertilizer media directly, and we used the media based on our previous study about technical media in laboratory scale. The result showed that this technical media can substituted Zarrouk Medium for growing Spirulina biomass in more cheap medium than ZM.
3	In Table 1 and 2: Salinity 10,20 and 30% means 100,200 and 30 grams per liter is very high and this under stress which means the reduction of growth rate.	°/ <sub>00</sub> mean part per thousand, it is not percent (%), so they are still range of salinity between fresh water (0 ppt), brackish water (5-20 ppt) and sea water (more than 25 ppt). The salinity used in this study still supports the

		growth of Arthrospira. They can grow
		between 0-60 ppt (Vonshak, 2000)
4	How is this given growth rate?	The reason is the same of no 3. Thank
		you
5	What are the components of wastewater?	Total phosphorus 2,6 mg.L <sup>-1</sup> , total
		nitrogen 1,9 mg.L <sup>-1</sup> , total organic
		carbon 11,4 mg. $L^{-1}$ : the source of N,
		P, C for growing
6	Figures 4 is not clear, should be redrawn for	We are very sorry, because we don't
	high quality.	have the high quality picture
7	Attention to references	We had corrected the references.
		Thank you.
8	There are some notes in the text.	We had corrected based on some notes
		in the text. Thank you

## Bukti konfirmasi artikel accepted (1 Desember 2020)





## Bukti konfirmasi artikel published online (1 Desember 2020)

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# Characterization of Arthrospira platensis cultured in wastewater of Clarias catfish farming media: DNA barcode, helical form, growth, and phycocyanin

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Abstract, Wijavanti M, Svafudin M, Yulisman, Nurianti Y, Hidavani A, Gofar N, 2020. Characterization of Arthrospira platensis cultured in wastewater of Clarins catfish farming media: DNA barcode, helical form, growth, and phycocyanin. Biodiversitas 21: 5872-5883. Arthrospira production technology in catfish waste media can be an alternative to reduce environmental pollution. However, some environmental factors such as nutrition, light, and water content can influence characterization of Arthrospira at the genetic and physiologic level, Arthrospira platensis is one of the phycocyanin-producing cyanobacteria and can be cultured using catfish culture wastewater. Water quality especially pH and salinity can effect of growth rate and residue of phycocyanin from Arthrospira platensis. This study aimed to identify the species and morphological forms of Arthrospira cultured using technical fertilizer and waste media, as well as to know the phylogenetic trees between species in this study and the GeneBank based on the 16S rRNA gene, and determine the optimum of pH and salinity required in the medium of catfish culture wastewater to phycocyanin maximum production of Arthrospira. The optimation of pH and salinity method used Completely Randomized Design (CRD) factorial with 2 factors consisting of the first factor with 3 treatments and the second factor with 4 treatments and 3 replications. The first factor was pH of culture medium i.e. pH 6.5 ± 0.2, pH 8.5 ± 0.2 and pH 10.5 ± 0.2. The second factor was salinity of culture medium, that was salinity 0 ppt (parts per thousand)‰), 10 ppt, 20 ppt, and 30 ppt. Parameters observed in Arthrospira include density, growth rate, rendement of phycocyanin, and decreased total nitrogen and phosphate content in culture media. The results showed that morphology Arthrospira cultured on technical fertilizer media (AF) had a longer and helix filament compared to Arthrospira cultured on waste media (AW) which showed several linear and shorter filaments. Both samples have a genetic distance of 0.068 (6.8%). Phylogenetic trees indicated that AF had a close relationship with Arthrospira platensis petH from Japan (bootstrap value 95%). While AW formed a separate sub-cluster of AF isolates and Arthrospira platensis petH from Japan (bootstrap value of 85%). The best treatment in this study was P2S3 (pH 8.5 ± 0.2 with salinity 20 ppt), which produced 0.867 grams maximum density, growth rate of 22.026 %, day-1 and 11.347 mg.g1 rendement of phycocyanin.

Keywords: 16S rRNA, Arthrospira, catfish culture wastewater, DNA barcode, pH, phycocyanin, phylogenetic analysis, salinity, Spirulina

## INTRODUCTION

Arthrospira is a genus of cyanobacterial microalgae, commonly known under the taxonomically incorrect brand name 'Spirulina' (Papapanagiotou and Gkelis 2019). The cyanobacterial genus Arthrospira Stizenberger ex Gomont 1892 contains at present 23 species, along with 12 infraspecific taxa (Guiry and Guiry 2010). They have variety characteristics of molecular, morphology, and physiology that based on polyphasic approach. Various genotypes are adaptable to various specialized ecosystems. The combination of different methods should be based on molecular sequencing as the basic approach, to which must be added other criteria (morphological, ecological) if they are available and which are distinct and recognizable in cyanobacterial populations. A polyphasic approach to include all the criteria obtained from morphological, biochemical, molecular studies, and phylogenetic to understand cyanobacterial classification as like as Arthrospira classification (Komárek 2018).

Recent studies have shown that Arthrospira can be used for treating wastewater, including effluent from fish culture, because the biomass can metabolize the nutrients and remove the pollutant from aquaculture effluent efficiently (Zhang et al. 2019). Industrial and processing wastes and by-products for culturing Spirulina (Arthrospira) are also being considered as alternative culture media, as like as aquaculture wastewater (Wijayanti et al. 2018; Widyantoro et al. 2018; Ragaza et al. 2020). Aquaculture could apply an integrated strategy of simultaneously treating aquaculture effluent while producing the biomass to supplement fish diets. The nutrient composition in their biomass depends on their environmental factor for growing biomass. Their character could be different from the various media for growth.

Basically, Arthrospira's morphology is characterized by trichomes that circular regularly (helical). However, abnormal morphology can also occur in Arthrospira as a circular shape that is irregular even linear. In some cultivation conditions, linear filaments can spontaneously return to the helix. However, there are significant differences in morphology, ultrastructure, physiology, biochemistry, and genetic characteristics between the original filament and the linear filament but not the difference between the original and the returned filament. Linearization in Arthrospira is a variation on the genetic level that can be caused by several environmental factors such as nutrition, light, and content of water media for growth (Wang and Zhao 2005). According to Liu et al. (2016), DNA barcoding has developed as a reliable technology for identifying species based on variations in the sequence of standard DNA regions. This method is used successfully in a variety of biological applications including finding cryptic species, detecting invasive species, and identifying plants. DNA barcoding is a simple short genome sequence amplified via PCR using appropriate primers (Adamowicz 2015). DNA barcoding using the 16S rRNA gene has been widely used to determine bacterial DNA characterization. Therefore, identification of Arthrospira using the 16S rRNA gene needs to be done to get the characterization of Arthrospira that is cultured on technical fertilizer and waste media and determine the phylogenetic tree structure that has been recorded in GeneBank.

Culture of Arthrospira (Spirulina) in Clarias pond farming wasted water could have specific characterization for optimal pH value and salinity. Their adaptation to grow in organic wastewater makes change in bioactive and important compound production. Their biomass has a nutritional value of 55-70% protein, 6-10% lipid 20% carbohydrate, besides being rich in minerals, vitamins, and pigments (Borowitzka et al. 2016; Vernes et al. 2015). Some color pigments that can be produced such as phycocyanin (blue pigment), allophycocyanin (blue-green), and phycoerythrin (red pigment) (Sharma and Tiwari 2011; Vernes et al. 2015). Phycocyanin is pigment in Arthrospira which has functions as an antioxidant (Pirenantyo and Limantara 2008), a source of food coloring, cosmetics, pharmaceuticals, and drugs (Tang et al. 2020; Tiwari and Tiwari 2020), anti-inflammatory, antioxidative and anticancer (Liu et al. 2013). One of the factors that influence phycocyanin levels is biomass (Taufiqurrahmi et al. 2017). The pH and salinity of culture media can affect the biomass of Arthrospira (Ciferri 1983; Marek et al. 1987; Planes et al. 2002). Ismaiel et al. (2016) showed that the diversity of the chemical composition of biomass is influenced by the pH of the growth media. Value of pH and environmental factors, especially salinity, influence the productivity of cell biomass, photosynthesis, shape, and flow of cellular metabolic activity that affect the dynamics of cell composition (Hu 2004). The optimal pH value for growth of Arthrospira sp is 7-10.5 (Hariyati 2008), and salinity from 15-30% (Thajuddin and Subramanian 2005). The salinity and pH value of Arthrospira culture media have been known to affect the morphology of the filament.

The aims of this study are characterizing morphological forms and DNA barcode based on the 16S rRNA gene of *Arthrospira* (*Spirulina*) cultured in fertilizer and waste water effluent of *Clarias* pond farming media, and determining optimal pH value and salinity of culture media for growth and phycocyanin production, especially in Clarias pond farming waste water media and morphological changes of their filament.

## MATERIALS AND METHODS

### Arthrospira cultured in agar media

Bacto agar was weighed as much as 2 g dissolved in 100 mL of water. The water used was swamp water and catfish culture waste that has been filtered and sterilized using an autoclave. Sterilized swamp water was then added with 0.02 gram MgSO4 fertilizer; CaCl2 0.004 gram; EDTA 0.008 gram; urea 0.03 gram; ZA (Sulphate of Ammonia) 0.132 grams; 0.4 gram baking soda; AB solution 1 mL mix A solution (Calcium Nitrate 64.26%, Potassium Nitrate 33.66%, Fe EDTA 2.08%) 2 grams / 10 mL and B solution (Potassium dihydro phosphate 25.83%, Ammonium sulfate 9.41%, Potassium sulfate 2.78%, Magnesium sulfate 60.91%, Cupric sulfate 0.03%, Zinc sulfate 0.12%, Boric acid 0.31%, Manganese sulfate 0.62%, Ammonium heptamolybdate 0.01%) 2 grams/10 mL water and TSP (Triple Super Phosphate) 0.05 grams were then homogeneous using magnetic stirrers. Next, wasted water was sterilized by an autoclave then cooled. Bactoagar was added to the technical fertilizer and waste solution to be homogenized using a magnetic stirrer and then boiled using a hot plate until all the ingredients dissolve and then autoclave again. The agar media was made with a pH of 7 and a salinity of 10 ppt or 10 g.L.1 (Hidayani et al. 2019).

Arthrospira cultivated in liquid media was taken 100 µL using micropipette and spread to the surface of a petri dish containing bactoagar media by using a sterilized spreader rod. Petri dishes were wrapped in wrapping plastic and then given a lamp lighting (light intensity 2000-4000 lux) with a dark: light ratio = 0: 24 hours. Arthrospira was observed every day until it grows blue-green. After growing, Arthrospira was re-cultured in agar media by the 4 quadrant scratch method. The cultures were used as isolate samples for determining DNA barcodes. The biomass of Arthrospira was isolated from commercial Spirulina TopSpira East Jakarta, Indonesia.

The commercial Arthrospira was cultured in technical fertilizer media directly, and we used the media based on our previous study about fertilizer media on laboratory scale (Laboratorium of Aquaculture, Faculty of Agriculture, Sriwijaya University). The result showed that this fertilizer media can substitute Zarrouk Medium (ZM) for growing Spirulina biomass in cheaper medium than ZM (Wijayanti et al. 2018).

### **DNA** extraction

DNA extraction was carried out according to procedures in which there was a Presto TM Mini gDNA Bacteria Kit (Geneaid Biotech Ltd.). DNA extraction consisted of several stages: sample preparation, lysis, purification, and precipitation or washing. The sample used was 0.15 grams of wet weight for one extraction (Geneaid manual).

BNA amplification The process of DNA amplification using the IVCR (Polymenase Cham Booktoni ineliad van performed innig 2 al. Envolute Jimmen BU (PCACOGC TAA CAC ACTO CAA GTC-3 <sup>+</sup> and mosce prime 1187° (P-GGG CDG GMT GTA CAA GGC 3 <sup>+</sup> (Matchole et al. 1986). The total composition of the PCR mattain usa 50 µL which consisted of 23 gL GF Tag Green, T3 µL NWR (Plackaue Proc. Watery and 8 µL derivolution DNA extinction regimen DNA amplification use strend out in stage. The initiation cycle at 979° C for 5 minutes, followed by 30 demamming cycle at 970° C for 5 minutes, followed by 30 SC for 30 seconds, then the extension stage at 72 °C for 1 minutes, and the final stage T2 °C for 7 minutes-(Lee et al. 2003).

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Figure 1. Cattion Income poord

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### RESULTS AND DISCUSSION

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Density and Growth Rate of Archrospira caltured in wastewater of cetfish farming. The biomess of Arthrospire displayed mechanism of

water ware achieved on a different day. The daily density of Arthroppine during influre can be seen in Figure 5.

The graph presented in Figure 5, it shows that in the sultrar period from day 1 to day 2. Arthropoto in noch immirrer-operimed slow growth, because the cells were still adapting to their new environment. The exponential phane for the treatment of P252, P253, and P551 hais from day 1 to day 8 of the exitors pariod. The immirrent of P251, P256, P251 and P251 taking phase from 2<sup>16</sup> went 8<sup>16</sup> day of the culture period. Walle for treatment of P151, P152, P153, P164, and P256 hais from 2<sup>16</sup> - 2<sup>16</sup> day of the valuer period. The deermaning definespine density for the treatment and P250 taking from 2<sup>16</sup> - 2<sup>16</sup> day of the culture period. The deermaning definespine density for the treatment occurred from day 8 to day 11 of the ruber. Learnam et al. [2019) rogitized that the adaptation phase lasts from day 0 to day 9 thes even the death phase after the 7<sup>16</sup> day and 9<sup>17</sup> day of these even the death phase after the 7<sup>18</sup> day and 9<sup>16</sup> day 10 the adaptation the and 9<sup>17</sup> the day 9 to day 9 thes even the data man. Sonis and Christian data and 9<sup>17</sup> day of the adaptation the taking and 9<sup>17</sup> day 10 the adaptation phase to a 2<sup>17</sup> day of the adaptation phase the 7<sup>18</sup> day and 9<sup>17</sup> day 10 the adaptation phase the 7<sup>18</sup> day and 9<sup>17</sup> day 10 the adaptation phase the 7<sup>18</sup> day and 9<sup>17</sup> day 10 the deermane of damsity could because of readaptating the adaptation phase the 1<sup>18</sup> days 1<sup>18</sup> data to the data



tia analysis with 1900 bootstop AW (Artheogenes cultured in westernoter reache) and AF (Artheogenes cultured in Figure 4, Phylager



Figure 5. Cell density (dry weight with a montage comm of 1.2%) of defensition in carllely teating need wanterware

The maximum density of hydrogene and the achieved an different days, between 5-1 days after collaring. The mean of manimum density reads to 0.453.0.307 g L<sup>2</sup> of days because collisional in catific teaching the college days and the day of the day of the teaching teaching the first vestry waveleners, readed in the production of 0.22 g L<sup>2</sup> of day because and analyzing productionly of 0.001 g L<sup>2</sup> day' (Requires et al. 2018). The catific muring post vestrements that high potential as collocation much for delying any days of the days of the days of the days of the second second second second second second second second vestrements that high potential as collocation much for delying size production.

ventrematrix that kiph potential as calibration multia for delenging propheticing. The second data differences in pild-ial interaction of vertices delenging and a districtly algorithmently affect the maximum dimetry and growth rate of delenging potential to the angle of the LBD and maximum dimetry to an all growth rate sequentially were presented in Tables 1 and 2, LBD and the sequencing of delenging potential of the LBD and main facts and a difference in pild balance (1) and the sequence of delenging plasma in the P2 constance (pild 6.5 + 0.2); and P3 transmission (pill 6.5 + 0.2). Assuming the delenging plasma (pill 10.5 + 0.2). Assuming a Parameter of produced in marks with a pill 6.8 + 0.2), instant (2016), the high-hier harmans of developme plasmas is produced in marks with a pill of 8.5 + 0.2); mage, a pill range further from its optimal pill can reduce its growth rate. A low growth rate will also many low homes predictions. disting

prediction. Furthermore, the different solution induced factors showed that the maximum density and growth rate in transmust 35 tableuty of 20 % over significantly higher compared in 35 tableuty of 20 % (solution) of 10 % and \$4 tableuty of 20 % in transmuss. The 51 and 54 transmuss were not significantly different and were the reasonable the produced the lowest density compared to other transmess.

Table I. Maximum domity of Arthrepoles platemic (g.l.\*).

10.1 Obveying a match name good names.
11.2 Different men 2: downed that the highest density and all performance of the second secon

Single INFLUENCE of	Single influence of solicity (S) (1.5D an = 0.107)				Main influence of pH (P)	
per (25)	51.18 %**	52100 542	53128 54	S4 (36 No	(1.50 par 4.85.5)	
P1 (p0 6.5) P2 (p0 8.5) P3 (p0 10.5)	0.435* 0.567*#0 0.667*#	0.033* 9.835* 0.733**	0.792 old 0.9957 <sup>2</sup> 0.763 old	0.407 * 0.309 *** 0.708 ***	0.075 <sup>3</sup> 0.817* 0.717 <sup>5</sup>	
Main influence of solution - (\$111,5D un=0.0625	0.623 *	8.735*	0.800 *	DOUGH *		

Table 3. The provili new	at at the copy of platerois.	onthe try a triaded	without y common	em ("L-by")
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Single Influence of pH (P)		Single linfle (LSI	Main influence of pH (P)			
	51 (0 %a)	ST (0 %) S2 (10 %) \$3 (20 %)		\$4(39)%4	- (LS0 (47-1,813)	
P1 (pl14.5) P2 (pl18.5) P3 (pl110.5)	14.031* 16.143*** 20.021**	18,785.0 20,559 ** 19,327**	22.010-1 22.010-1	25.348* 30.725* 36.417*	16, 363 * 20, 416 * 19, 147 *	
Main influence of saliony-	17.395*	19:3857	20.014.9	101.825 *		

Note: 5% means part per Bananal (ppf), it is not person (%). Balanky of Bachmain (0 ppt), brackah sain (5-20 ppt), and mix value source than 25 ppt). The salarity and to this study still support the growth of Antinescence (Visiolask 2007).

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Table X. Physiocynene (%) yield in Arthrophys day beeness at 8 days after inscidution

Single Influence of pill (P)		Single infl (LS	Main influence of pH (P)		
	SI	52	\$3	54	0.50 05*0.096
191 192 193	7.880 * 9.657% 8.070*	8,783 * 39,995 * 14,406 *	0.441 <sup>4</sup> 11.347 <sup>1</sup> 10.134 <sup>1</sup>	8.3673 10.423+ 4.262+	8.625 <sup>+</sup> 10.585 <sup>+</sup> 0.448 <sup>+</sup>
Main influence of adapty (StickSD are=0.111)	8.016.*	8.608*	10.367.0	8.3577	

A	Sec. 21	1		6 - A - A - S - S - S - S - S - S - S - S	12 C 1		and the second	
Table 4, III	124/2010/01/01	Fal 431-	WINDOW UND	La JanMares	110.00	Access of the	Allen ("NA	

Single Inflaence of pill (P)	1979	Single in (L	Main influence of pl (P)		
2	51	82	83	54	(1.50 sec-0.645)
PL	30.998	10:250 #	\$5.767 ····	\$1.801 M	82.226*
12	33.882.**	84.377.49	85.420*	84.857 H	84.633 *
P3	82.840 <sup>4vel</sup>	81.141 *	84.9382*1	64303 P	83.402.*
Main influence of Solitity (S1(3)SD caref(343)	\$2.5811	163.856 h	84.712*	62.601*	

The mechanism for removing ritrigger in water is determined by soveral factors, including bacturial activity (Geodesig et al. 1986), uptake by plants (Bross 1990), and expertise of the Sancher-Moneelee et al. 2001). In Speedbactura, minopan is a macrosurient that play an important rule in the formation of functionatic composatio and a medicis social (DNA, RNA), anime solid (promi-ing) plants induced by the solid solid solid solid solid an medicis social (DNA, RNA), anime solid (promi-sion) plants induce real is in the solid theory of the solid solid solid solid solid solid theory of the solid solid solid solid solid solid theory of the solid solid solid solid solid solid robospice automousles in transmit P2 compared to order tourisets, so that the utilitation of netroger by developeins on collarse medicis is gradies that is of netroger by developeins on collarse medicis is gradiest then is offers, Marking at (2014) theored that the fugine size and the isolating nitrogen. including nitrogen. The value of reducing total nitrogen content in \$3.

Including subcogen. The subset of reducing total netrogen centersi in S5-transmus was significantly higher than for other transmosts. This showed that transmust S5 caused televospics to absorb introgen higher than other treamostis in the with the high durative and menisterior of physosystems televospics observed in this staty. Jahoen and Ahrend (2011) showed that salisity on celture media influences introgen absorption and protein biosynthesis. Robustion of initiation of that salisity on celture media influences transmostic biosynthesis. Bologen thesis influences transmostic biosynthesis and analytic pattern in the same from the higher density, powers does not renderesent of physoceparis faund in trustment PSSs. The amount of intera and physical descenses with merunaniag grandsh of Chloredia valgaria and Artimopius pharenels (Stepali et al. 2016). This to because algar have the density physical and a sitting and physical and a stratisty. The amount of intera and physical descenses with merunang grandsh of Chloredia valgaria and Artimopius pharenels (Stepali et al. 2016). This to because algar have the density to show huminest with an introgen and physical are used to carry and physicaphics and provide grands.

Reduction of phosphorus contant in waters is influenced by the process of absorption, complexation, deposition and assumilation (between microbes and plant hiomasis) (Tamar et al. 19993.

The results of LSD set on the main factor of pH showed that each transmut data to significantly afford the relation of phraphare sonant it is driven by afford the relation of phraphare sonant it is driven by a set of H<sub>2</sub>PO<sub>3</sub> and HPA<sub>2</sub><sup>-1</sup> face of traphosphare is non-like perturbation. Plant with the set of the set of the set of H<sub>2</sub>PO<sub>3</sub> and HPA<sub>2</sub><sup>-1</sup> face of traphosphare is non-like perturbation. The orthophosphare content decreases with increasing media pH. Crease and Fitzianeous (2016) showed the orthophosphare content increases in the pH maps face in 5.5 and decreases when pH 10. The fall in the entrophosphare content at pH 16 is due to an increase in calcian phosphare formation. The value of reducing the phosphare resist in derivative solution of different advects. The solution factor. The results of LSD and on the main factor of pH show

phosphase context in divisory or cubase resolute was presented in Table 5. The solution Table 5. The solution factors, advancements of different solution in tach treatment has an significant effect on contextuors cor-tication of phospherus will be ublifted when a combination of CI and already and Concornation cor-tication of CI and already and Concornation cor-tication of CI and already and Concornation of the additional solution, when the solution of the con-text of CI and already and Concornation of the relation of CI and already and Concornation cor-tication of CI and already and the solution of the solution of CI and already and the solution of the solution and alleady socie treatment and no significance (EGP) can be triangly active speake and call of the solution of the context framework and the solution of the solution of the triangle active speake and call of the solution of the DOF assignments for all the solution of the solution of the triangle active speake and the solution. Markow of all (2014) stand that phospherus as a macrossentium that plays as important role in the properation of nucleis and (RNA and DNA), phospholiption and energy-service markeesile (LTP). The phospherus nucleis and the solution of variance shows that the results are not assignificantly different, but the highest phosphate rolucions of the assignificantian (225).

<text><text><text><text><text><text><text><text>

Advances of 0.005 g L ' (Preack 2019). Bergen The State of the State of the State of State

Reduction of total altreges and phosphate content The percentage twinetion of total mitrages in the estime coolist was presented in Table 4.

WEAS ANTLAS all - Arthropping glassials cultured in matter rater of Classic cutter for gaming marks 5881

Table 5. Roberton of phosphato control in Archerging callure molecus (%)

Stands and standard states and	- 002 - L	Single influence	e of salisity (N)	212 2	Adventure of the second second second second
Single subsceece (60 (F)	58	52	83	54	- And an interact or fair (a)
P1	70.588	71.667	T1.000	72,583	71.875
192	76.067	74.i00	74.007	72,067	"Acceleti
#3	791,218.0	10.318	T1 007	75,067	71,756
Many reflection of subsite (Sy-	71.815	73 800	73.779	72,996	



Figure 4. Marghology of detherquine in onlich matemate collace mode in second instruments of philand solution

### Murphology of Asthrospire in various pH and solinity

netia Mrephology of derivergene was affected by increasing or decreasing physical or chemical factors in their rather media. Solitely and acadity take constraints at this study due't sharper of derivergene agriculture of the study of entropy of derivergene and emissioncope with 10m at future of derivergene and emissioncope with 10m at future of derivergene and emissioncope with 10m at future of the source of a different in all pill and solidity transmitt. The source oper study poly and solid trans of affected in the hearing filterent. This most reduced at the study reduced of the study r

that salisity and acidity of output modes didn't affect

that solicity and addyr of adhrer arefus fields affinet seriana frees of flavores, effect straight and heined. The atnapht Blannards were observed for Arrivoyne trains during two years of calibrations, and hein preserve it Ardrospine an Neglin CL calibrars was constant. The printer merghanized planticity, gravity influences by the provide stage and reagonase permeters, each an emperature and light influencity, was observed for Artinopero ensite (Engapsingiants and Occilia 2019). There are indications that adaptivity to change an emissionmental somilities in relatively rapid and also

subsequent changes at the genetic level can be realized quickly. This means that we can easily find different genotypes in various stable, ecologically different habitats. Morphologically similar strains were cultured for a long time under uniform and stable conditions (Komarek 2016). But the morphological changes could not be effected by acidity and salinity of culture media.

In conclusion, Arthrospira that is cultured on waste media (observed in liquid culture) indicated some short and linear filaments. Identified Arthrospira had a genetic distance of 6.8% between AF and AW isolates. AF isolates had a close relationship with Arthrospira platensis petH species originating from Japan (bootstrap value of 95%) while AW isolates form phylogenetic branches which are separated from AF isolates and Arthrospira platensis petH species originating from Japan (bootstrap value 85%). The catfish culture wastewater media at different pH and salinity affects the density, growth rate, and rendement of phycocyanin Arthrospira platensis. The highest density, growth rate and rendement of phycocyanin were in P2S3 treatment (pH 8.5 ± 0.2 and salinity of 20 ‰) which produced a maximum density of 0.867 g L-1, growth rate of 22.026% day1 and the rendement of phycocyanin of 11.334 %.

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