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Evaluation of bio-resources: monitoring *Arthrospira* growth in supplemented brackish water

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SUMMARY. *Arthrospira* is a cyanobacterium with high nutrient values that is mono cultured on a commercial scale. A new empirical growth medium was formulated by incorporating selected nutrients of the standard Zarrouk's medium in natural brackish water. Growth of *Arthrospira* in this medium was observed on the basis of protein and pigment contents and microscopic examination. Results sustain the use of this nutrient medium as cost effective method for economically important cyanobacterium cultivation, furthermore possible contamination risks were identified.

Keywords: *Arthrospira*, brackish water, chlorophyll, growth

Introduction

Arthrospira fusiformis Woronichin is a species of planktonic photosynthetic filamentous cyanobacterium (Vonshak *et al*., 1988, Lefort *et al*., 2014). *Arthrospira* cells present a high nutritive value (contain essential nutrients: proteins, minerals, provitamins, polyunsaturated fatty acids), therefore are widely used as food supplements (Belay, 1993; Ogato and Kifle, 2014). Due to its ability to tolerate a wide range of salinity levels, a good quality monoculture can be achieved and competitor growth can be limited by regulating salt concentrations (Zeng and Vonshak, 1998; Ahmed *et al*., 2014). A major drawback of commercial scale production is the high cost of growth medium (Raoof *et al*., 2006; Mahrouqi *et al*., 2015). Thus, our future aim is to modify and accommodate a natural brackish water basin, for cost effective *Arthrospira* production in outdoor pond system. This paper focuses on growth monitoring of two *Arthrospira* strains in supplemented brackish water, on the biodiversity shift induced by *Arthropsira* in this natural habitat, and which species can compete with *Arthrospira*, contaminating the mono culture.

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Materials and methods

Water from an alkaline basin in Dezmir (approx. surface area of 400 m^2, pH of 8.57-9.11, salinity 21.2-26.6 g/L) was filtered, chemically analyzed and supplemented with C, N and P to develop an empirical, nutrient enriched growth medium (EGM) that resembles Zarrouk (Z) medium, typically used for *Arthrospira*. Strains were provided as *Arthrospira platensis* AICB 49 isolated from Egypt (growth rate comparable to those used in industrial production) (Fig. 1 a) and *Arthrospira fusiformis* AICB 606 (isolated from an alkaline lake, Cluj County) (Fig. 1 b-c) by AICB (Culture Collection of Algae of the Institute of Biological Research - Dragoș et al., 1997). For the experiments parallel series of different combinations of Z medium, EGM, one of the *Arthrospira* strains, and homogenized mat were made. Series were cultured in 3 replicates, in volumes of 200 ml, at room temperature (22-24°*C*), under constant illumination (315 $\mu mol \cdot m^2 \cdot s^{-1}$), for 14 days. Samples were collected daily for specific growth rate parameters: pH, optical density (678 nm) and chlorophyll *a*. Initial and final analysis included dry weight measurements, chlorophyll *a*, *b*, *c* and total chlorophyll determinations (Ritchie, 2006; Ritchie, 2008; Keresztes *et al*., 2010). Taxa were identified from enrichment cultures by optical and electron microscopy examinations (Ettl and Gärtner, 2014).

Results and discussion

The EGM, developed by complementing brackish water with anions (HCO_3^-, NO_3^-, PO_4^{3-}), can sustain the growth of *Arthrospira* at a rate comparable or even higher than standard growth medium. This was supported by the daily chlorophyll *a* concentrations (μg/ml) and biomass dry weight (mg/L) measured after 14 days. Growth curves, based on optical density measurements, showed a slower growth start than in case of Z medium, and also a delayed stationary phase. AICB 606 strain had a higher growth rate both in EGM and Z compared to AICB 49 strain, also higher dry weight at the end of experiments, 563.88 mg/L in EGM and 450.81 mg/L in Z compared to values of AICB 49, 439.78 mg/L in EGM and 206.24 mg/L in Z respectively, suggesting that AICB 606 is pre-adapted to the specific chemistry of this area.

Growth of *Arthrospira* as dominant photosynthetic microorganism, led to appreciable increase in pH (from the initial 8.6-9.1 up to 11) and implicitly to reduction of the native flora that is a potential growth competitor, therefore a biomass contaminant. Initially, a total of 33 taxons were identified in the alkaline water basin. Cyanobacteria dominated the habitat (15 taxa), followed by diatoms (8 taxa) and green algae (3 taxa) (Fig. 2). The risk of monoculture contamination was assessed by measuring chlorophyll *a, b* and *c* levels in the biomass. This allows to distinguish between cyanobacteria (containing only chlorophyll *a*), green algae (containing chlorophylls *a* and *b*) and other groups (that also contain chlorophyll *c1* and *c2*).

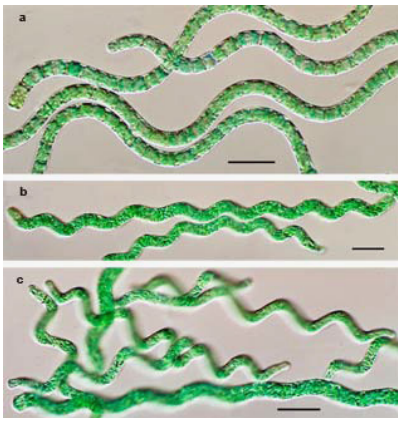

Figure 1. a- *Arthrospira platensis* AICB 49;
b-c – *Arthrospira fusiformis* AICB 606. Bar dimension: 20 μm.

Low chlorophyll *c* values indicate that there is no notable risk of contamination of the biomass, although *Chlorella homosphaera* (containing chlorophyll *a* and *b*) seems to be capable to grow both in EGM and Z. Eight cyanobacterial taxa were detected in the final experimental culture, revealing that they are an evident risk factor even if their presence cannot be distinguished by chlorophyll measurements. Five species were identified as possible competitors of AICB 606, in the EGM: *Synechocystis crassa*, *Synechocystis salina*, *Synechococcus* sp., *Oscillatoria limnetica* and *Pseudanabaena minima.*

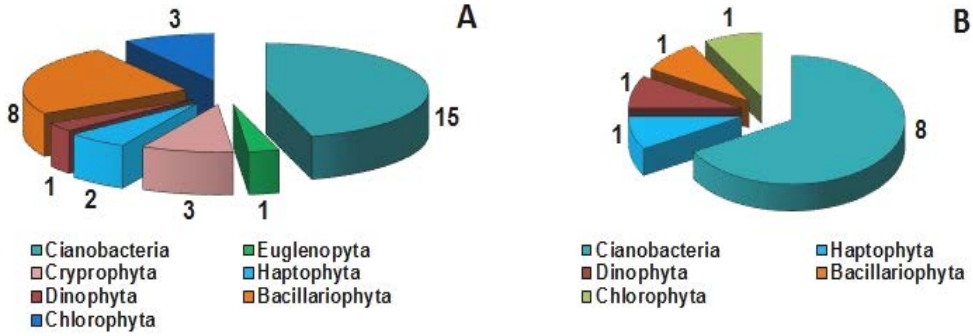

Figure 2. Total number of phyla and taxa identified in the brackish Dezmir basin (**A**) and in the experimental cultures (**B**)

Conclusions

A new empirical growth medium was developed that can support a rapid and constant growth of *Arthrospira* at levels comparable or even higher than standard nutrient medium, AICB 606 yielding higher growth rates in every experimental condition than AICB 49. Among potentially contaminating taxa, *Synechocystis crassa*, *Synechocystis salina* (cyanobacteria) and *Chlorella homosphaera* (green algae) have high probability of impeding the establishment of a stable monoculture.

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REFERENCES

Ahmed, M., Moerdijk-Poortvliet, T., Wijnholds, A., Stal, L. J., Hasnain, S. (2014) Isolation, characterization and localization of extracellular polymeric substances from the cyanobacterium *Arthrospira platensis* strain MMG-9, *Eur. J. Phycol.*, **49**(2), 143–150

Belay, A., Yishimichi, O., Kazuyuki, M., Hidenori S. (1993) Current knowledge on potential health benefits of *Spirulina*, *J. of Appl. Phy.*, **5**, 235-241

Dragoș, N., Péterfi, L. Ș., Momeu, L., Popescu, C. (1997) *An introduction to the algae and the culture collection of algae at the Institute of Biological Research*, Cluj University Press, Cluj-Napoca

Ettl, H., Gärtner, G. (2014) *Syllabus der Boden-, Luft- und Flechtenalgen*, 2nd Edition, Springer Spektrum, Berlin, pp 773

Lefort, F., Calmin, G., Crovadore, J., Falquet, J., Hurni, J. P., Osteras, M., Haldemann, F., Farinelli, L. (2014) Whole-genome shotgun sequence of *Arthrospira Platensis* strain paraca, a cultivated and edible cyanobacterium, *Genome Announc.*, **2**(4), e00751-14

Mahrouqi, H. A., Naqqiuddin, M. A., Achankunju, J., Omar, H., Ismail A. (2015) Different salinity effects on the mass cultivation of *Spirulina* (*Arthrospira platensis*) under sheltered outdoor conditions in Oman and Malaysia, *J. Algal Biomass Utln.*, **6**, 1-14

Ogato, T., Kifle, D. (2014) Morphological variability of *Arthrospira (Spirulina) fusiformis* (Cyanophyta) in relation to environmental variables in the tropical soda lake Chitu, Ethiopia, *Hydrobiologia*, **738**, 21–33

Raoof, B., Kaushik, B. D., Prasanna, R. (2006) Formulation of a low-cost medium for mass production of *Spirulina*, *Biomass Bioenerg.*, **30**, 537-542

Ritchie, R. J. (2006) Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents, *Photosynth. Res.*, **89**, 27-41

Ritchie, R. J. (2008) Universal chlorophyll equations for estimating chlorophylls *a, b, c,* and *d* and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents, *Photosynthetica*, **46**, 115-126

Vonshak, A., Guy, R., Guy, M. (1988) The response of the filamentous cyanobacterium *Spirulina platensis* to salt stress, *Arch. Microbiol.*, **150**, 417–420

Zeng, M. T., Vonshak, A. (1998) Adaptation of *Spirulina platensis* to salinity-stress, *Comp. Biochem. Physiol.*, **120**, 113–118