Int.J.Curr.Microbiol.App.Sci (2016) *5*(10): 233-239

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 10 (2016) pp. 233-239**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.510.025

Antioxidant Effect of Phycobiliproteins of the Cyanobacteria *Arthrospira maxima* on Growth of *Saccharomyces cerevisiae* under Oxidative Stress

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Keywords

Antioxidants, phycobiliproteins, Arthrospira maxima, Saccharomyces cerevisiae.

Article Info

Accepted:
12 September 2016
Available Online:
10 October 2016

A B S T R A C T

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the biological system capacity to either rapidly detoxify the precursor reagents, or to repair the resulting damage. All ROS are associated to several illnesses and health disorders like cancer, aterosclerosis, Alzheimer´s disease, diabetes and age related deterioration amongst others. The reason for that relies in the fact that ROS react with biomolecules such as lipids, proteins and DNA, and neutralise their function. Previous reports have proven that the phycobiliprotein extract of some species of algae and cyanobacteria acts as a free radical scavenger. The aim of this project was to observe the antioxidant effects of a raw phycobiliprotein extract of *Arthrospira maxima* in the survival of *Saccharomyces cerevisiae* previously submitted to oxidative stress through the formation of –OH radicals as result of the Fenton reaction, using ascorbic acid and Cu(II). *S. cerevisiae* strain was grown in agar Sabouraud, and then transferred to an oxidative stress treatments in presence and absence of raw phycobiliprotein extract of *Arthrospira maxima*. The results show that the survival of *S. cerevisiae* in dilutions of 10^{-2} and 10^{-3}, go from 7 X 10^{5} to zero in the presence or absence of the mentioned raw extract. Therefore, applying a non-parametric statistical test, a meaningful difference between treatments was obtained. All this led us to conclude that the presence of phycobiliproteins and their tetrapirrolic structure, reaction with ROS and enhances the growth of *Saccharomyces cerevisiae* in an oxidative media.

Introduction

Oxidative stress occurs when the balance of oxidants within the cell exceeds the levels of antioxidants. Increased levels of ROS can lead to the damage of macromolecules within the cell; this damage to lipids, proteins, and DNA can rise to pathological consequences. There are convincing evidences that oxidative stress and ROS play an important role in the etiology and progression of several human diseases (Bermejo-Bescos *et al.*, 2008). Antioxidants are needed to prevent the formation an oppose the actions of reactive oxygen and nitrogen species (Cervantes *et al.*, 2005; Riss *et al.*, 2007).

Phycobiliproteins are accessory photosynthetic pigments of cyanobacteria and red algae, are water soluble proteins attached covalently to tetrapyrrole prosthetic groups called phycobilins, are divided in three classes: phycoerithrins, phycocianins and allophycocianins (Bermejo *et al.,* 2003).

Phycobiliproteins from cyanobacteria has been used as antioxidants in some toxicological models, as well as fluorescent markers and as natural pigments in food industry (Glazer, 1994; Chamorro *et al.,* 2002).

Since their structure is similar to bilirubin, well known as an effective scavenger of ROS in blood, phycobiliproteins have gained an increasing reputation for its antioxidant potential. It has been shown that protein extracts from *Spirulina platensis* have a significant lowering effect of ROS (Patel *et al*., 2006), phycobiliproteins from *Pseudanabaena tenuis* can protect from damage caused to kidney by exposure to Hg^{2+} (Cano-Europa *et al.,* 2010), phycobiliproteins from *Spirulina maxima (Arthrospira maxima)*and *Pseudanabaena tenuis* protect against hepatic damage and oxidative stress caused by Hg^{2+}(Gallardo *et al.,* 2010). Phycocyanin is able to remove alkoxyl, peroxyl and *in vitro* hydroxyl radicals and inhibiting lipid microsomal peroxidation, among other activities; the protein extract of *Aphanizomenon flos-aquae* decreases oxidative hemolysis and lipid peroxidation in erythrocytes (Sekar and Chandramohan, 2007); and a phycobiliprotein extract from *Dermonema virens* protected *Lactuca sativa* seedlings in an oxidative stress assay (Ortiz-Garcia *et al.,* 2016).

Although there are some data supporting that the apo-protein is responsible for the antioxidant properties of FBP's, most studies considered that these properties are due to the chromophores. The similarity between them and bilirubin is remarkable, and the enzyme that reduces the biliverd in to bilirubin (which is the form that is considered antioxidant because it binds to albumin, protecting from ROS), the biliverdin reductase, also reduces the phycocyanobilin to phycocianorrubin (Eriksen, 2008).

Therefore, the aim of the present investigation was to show the antioxidant effect of phycobiliproteins extracted from *Arthrospira maxima* on the growth and viability of *Saccharomyces cerevisiae* in a ROS generator system.

Materials and Methods

Microorganisms culture

Saccharomyces cerevisiae was grown on Sabouraud agar at 28° C for 48 hs, an inoculum of this culture was suspended in 5 mL of Sabouraud broth and incubated at 28^{o}C for 48 hs, was adjusted with McFarland nephelometer and this culture was used for bioassay.

The cyanobacteria *A. maxima* was grown in Zarrouk's medium (Zarrouk, 1966), in batch cultures at 28 ^{o}C for 15 days in 1 L vessel, the cultures were aerated and illuminated with daylight fluorescent lamps using a 12/12 photoperiod. The biomass was harvested using Whatman paper No. 1.

Phycobiliproteins *extraction*

Biomass of *A. maxima* was suspended in phosphate buffer (0.1M, pH 7.0), then frozen and thawed three times, 1% streptomycin sulfate (p/v) was added, the slurry was refrigerated 24 hs and was centrifuged to remove cell debris and obtain

the crude extract. Spectroscopic analyses were carried out using a Shimadzu™ Spectrophotometer UV/Vis 2000 UV), reading at 650, 620 and 565 nm. Also, continuous absorption spectrum (400–700 nm) was collected using a 1 cm quartz cuvette. The emission intensity signal for the extraction buffer was subtracted from the values obtained from each sample. Emission spectra were obtained for every 5 nm. The extraction buffer was used as reference for phycobilins. The amount of C-PC, C-PE and C-APC in the sample was calculated using simultaneous equations and the extinction coefficients as follows (Bennet and Bogorad, 1973; Soni *et al.*, 2006):

PC (phycocyanin) = {A620nm– 0.7(A650nm)}/7.38

AP (allophycocyanin) = {A650nm– 0.19(A620nm)}/5.65

PE (phycoerythrin) = {A565nm– 2.8 (PC) – 1.34(AP)}/12.7

Phycobiliproteins and ROS assay on *S. cerevisiae* growth

Four bioassays were performed: 1) Control assay with 200 µL *S. cerevisiae* suspension and 400 µL saline solution (0.15 M); 2) ROS and PBP assay with 200 µL *S. cerevisiae* suspension,40 µL ascorbate sodium 100mM plus 2 µL copper sulfatum 10 mM, 158 µL saline solution and 100 µL PBP extract (sterile by filtration with 0.22 µm pore size filter); 3) ROS assay with 200 µL *S. cerevisiae* suspension, 40 µL ascorbate sodium 100mM plus 2 µL copper sulfatum 10 mM and 258 µL saline solution; and 4) PBP assay, with 200 µL *S. cerevisiae* suspension,200 µL saline solution and 100 µL PBP extract. The mixes were kept at 28ºC/24 hr in darkness. Then were diluted and 100 µl of 10^{-4} dilutions were spread over Sabouraud agar dishes, by duplicate, and were kept at 28º C for 48 hr.

The experiments were performed by octuplicate and after incubation, the colony forming units (CFU) were calculated.

Results and Discussion

Phycobiliprotein extraction

The table 1 shows phycobiliprotein extract's absorbances at 565, 620 and 650 nm and the concentrations for each phycobiliprotein (phycocyanin, allophycocyanin and phycoerythrin). The figure 1 show the phycobiliprotein extract's absorption spectrum, from 400-700 nm, the wavelength of maximum absorption (nm) is at 615 nmso, the phycobiliprotein found in higher proportion is the phycocyanin, which coincides with the concentrations obtained by substituting the values of Table 1 in the equations of Bennet and Bogorad (1973) and Soni *et al.*, (2006): phycocianin 0.224 mg/mL, allophycocyanin 0.113 mg/mL and phycoerythrin 0.34 mg/L.

Phycobiliproteins and ROS assay on *S. cerevisiae* growth

Table 2 shows the CFU calculated from the phycobiliproteins extract and oxidant media assay. Figure 2 shows four plates where *Saccharomyces cerevisiae* was tested in saline solution (T1), phycobiliproteins extract plus oxidant conditions (P1), just oxidant conditions (P2) and phycobiliproteins extract plus saline solution (P3).

The average colony forming units (CFU) in the eight plates T1 is 3 X 10^5, similar to the average CFU in the eight plates P1 and P3. But in P2 (ROS assay) the average CFU is 0 (zero).

Table.1 Phycobiliproteins concentration

Wavelength (λ) nm	Absorbance	Phycobiliprotein	Concentration (mg/mL)
565	0.121	PC	0.2235
620	0.235	AP	0.1130
650	0.121	PE	0.0341

Table.2 CFU calculated from ROS and PBP assays

Assay	AverageCFUcount
T1) *Saccharomycescerevisiae*, NaCl	3×10^5
P1) *Saccharomycescerevisiae*, NaCl, Na-ascorbate, $CuSO_4$, Phycobiliproteinextract	8×10^5
P2) *Saccharomycescerevisiae*, NaCl, Na-ascorbate, $CuSO_4$	0
P3) *Saccharomycescerevisiae*, NaCl, Phycobiliproteinextract	9×10^5

Fig.1 *Spirulina maxima* phycobiliprotein extract's absorption spectrum. Maximum at 620 nm, A=0.235

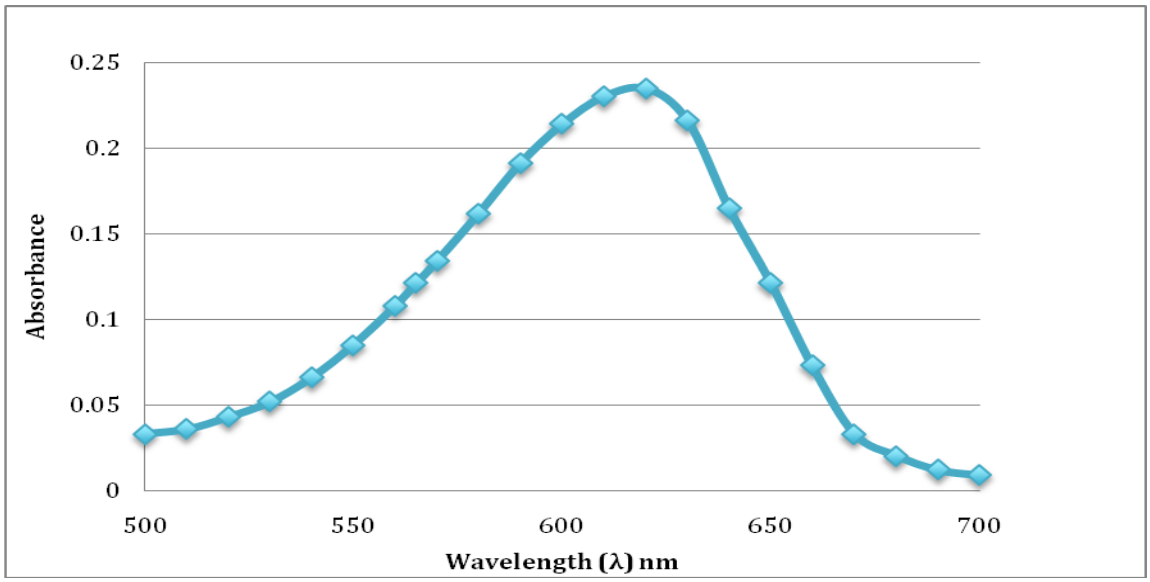
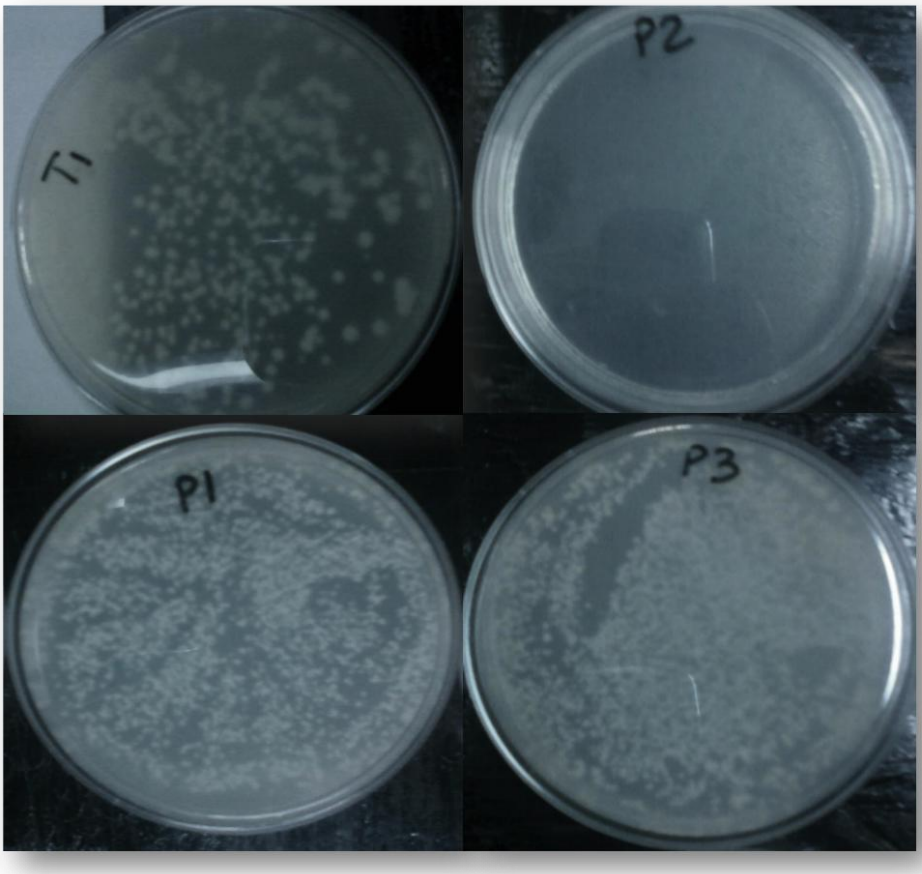


Fig.2 ROS and PBP assay results: T1) *Saccharomyces cerevisiae* and NaCl; P1) *Saccharomyces cerevisiae*, NaCl, Na-ascorbate, CuSO4 and phycobiliprotein extract; P2) *Saccharomyces cerevisiae*, NaCl, Na-ascorbate, and CuSO4; P3) *Saccharomyces cerevisiae*, NaCl and phycobiliprotein extract



Saccharomyces cerevisiae has the ability to respond to oxidative stress by generating a response at molecular level and inducing antioxidant defense systems. To counteract oxidative stress, signal transmission routes and specific factors are activated, and gene expression of antioxidant proteins grows. This specific response to oxidative stress has the function of decreasing levels of ROS and repair the damage caused by these oxidants, protecting cellular components (Folch-Mallol *et al.*, 2004), according to them, *S. cerevisiae* has some nonenzymaticsystems, like glutathione, that captures the most abundant reactive intermediates of oxygen in the cell thanks a sulfhydryl group which reacts with oxidants. However, when the oxidizing conditions increase, these regulatory pathways may be exceeded, so the presence of antioxidants is required, as would phycobiliproteins proposed in this paper.

The antioxidant activity is due the molecular structure of the phycobiliproteins, which gives them the ability to interact with ROS, neutralize them and prevent damage that can cause the cells (Gallardo *et al.*, 2010; Benedetti *et al.*, 2004; Patel *et al.*, 2006;Romay*et al.*, 2001; Zhou *et al.*, 2005).

Results of this work coincide with previous studies, in animal models, that demonstrate the antioxidant activity associated with the

content of phycocyanin in PBP extracts, therefore, the yeast model used shows to be a useful replacement to *in vivo*assays related to antioxidant activity (Riss*et al.*, 2007).

In conclusion, phycobiliproteins extract from *Arthrospira maxima* protected *Saccharomyces cerevisiae*in an oxidative stress assay: CFU were normal, even sorrounded by ROS.

Acknowledgements

Authors would like to thank the Research Project SIP: 20143986 of the Secretaría de Investigación y Posgrado del Instituto Politécnico Nacional, for providing the facilities to carry out this work and also would wish to thank the fellowships from Comisión de Operación y Fomento de Actividades Académicas (COFAA, I.P.N.) and EDD (Estímulo al DesempeñoDocente, I.P.N.).

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How to cite this article:

Irma Itzel Sánchez García, Nora Beatriz Medina Jaritz and Roxana Olvera Ramírez. 2016. Antioxidant Effect of Phycobiliproteins of the Cyanobacteria *Arthrospira maxima* on Growth of *Saccharomyces cerevisiae* under Oxidative Stress. *Int.J.Curr.Microbiol.App.Sci.* 5(10): 233-239. doi: http://dx.doi.org/10.20546/ijcmas.2016.510.025