EXTRACTION OF AGGLUTININS FROM *Arthrospira platensis* BIOMASS IN THREE DIFFERENT BUFFERS

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ABSTRACT – *Arthrospira platensis* contains large amounts of proteins, vitamins, lipids and pigments with various biological functions, such as antitumor, antiviral, antimicrobial activities. The aim of this work was detected the presence of agglutinins in *Arthrospira platensis* biomass extracted in three different buffers. The harvested cells were concentrated by a centrifugation at 4,000 *x*g for 10 min, lyophilized and extracted under sonication in pure and in concentrations of 1:5 to 1:20 of 0.025M Tris-HCl-saline, pH 7.0; 0.02M saline-phosphate, pH 7.4; and 0.1M sodium acetate, pH, 5.5 buffers. For hemagglutination activity tests, in microtiter boards, extracts were added to rabbit erythrocytes. Positive results were obtained for hemagglutination activity only in saline-phosphate buffer (1:5, 1:10, and 1:20 dilutions) and sodium acetate buffer (1:5, and 1:10 dilutions) with titers of 2^{12} and 2^{7}, respectively. These organisms would be potential sources of novel agglutinins for biomedical research.

1. INTRODUCTION

Cyanobacteria are prokaryotes that perform oxygenic photosynthesis and constitute a large taxonomic group within the domain of Eubacteria. Cyanobacteria are divided morphologically (unicellular or filamentous) or functionally (N_2-fixing and non-N_2-fixing). Filamentous species are subdivided into those with and without a heterocyst which is a differentiation from vegetative cells for fixing nitrogen (DESIKACHAR, 1973; DOOLITTLE, 1982). *Arthrospira* is a representative filamentous non-N_2- fixing cyanobacterium that lacks any differentiation such as for the heterocyst, akinete or hormogonium, which develops in some filamentous N_2-fixing cyanobacteria. This genus is also well known as '*Spirulina*' because of its useful property as a food. The Aztecs consumed it regularly (VAN TUERENHOUT, 2005).

Historically, the classification of *Arthrospira* and *Spirulina* genera was a subject of controversy. For the commercial strain, *Arthrospira* or *Spirulina* was used interchangeably. Both *Arthrospira* and *Spirulina* are similar in morphological characters, such as: cylindrical, multicellular, filamentous cyanobacteria with an open, left-handed and helical shape. They both belong to the Phylum *Cyanobacteria*, Order *Oscillatoriales* and Family *Oscillatoriaceae*. (CASTENHOLZ, 2001) However, those organisms can be differentiated by the presence of cell septa: *Arthrospira* possess septa, whereas *Spirulina* do not (TOMASELLI *et al*., 1996).

Arthrospira platensis shows vigorous gliding motility of filamentous cells (trichome) with rotation along their long axis. Gliding is a self-propulsion across a solid or semisolid material without the aid of any visible flagellum (HOICZYK, 2000) and it has become an important industrial organic material as a health supplement, a source of β-carotene and a natural colouring agent. The presence of hydrogenases in its cells also makes this cyanobacterium a useful material for clean energy production (LOSEVA and DARDYNSKAYA, 1993; AMAO and NAKAMURA, 2006).

Several studies have shown significant interest in finding compounds that have biological activities from microalgae biomass, one of applications of bioactive extracted by natural products is the hemagglutinating activity. Haemagglutinin are proteins with characteristics of binding to specific carbohydrate. These biomolecules are abundant in nature, are important for cell aggregation and glycoconjugates and consequently have a high relevance to biomedical and pharmaceutical research. Seaweed (macroalgae) hemagglutinins have the ability to agglutinate erythrocytes treated enzymatically preferably of rabbits and other animals. Several studies reported that hemagglutinin algae have immunomodulatory and antitumor activities *in vitro e in vivo* (LIMA *et al*., 1998; NEVES *et al*., 2001). This study aims to determine the best extraction method of possible hemagglutinins biomolecules using three different buffers through sonication to establish the hemagglutination activity of the extracts of *Arthrospira platensis*.

2. MATERIALS AND METHODS

2.1. Microalgae and media

Arthrospira platensis (UTEX 1926) was obtained from the UTEX (University of Texas, Austin). Cultures were maintained axenically in broth culture medium described by SCHLÖSSER (1982), composed (g/L): $NaHCO_3$, 13.61; Na_2CO_3, 4.03; K_2HPO_4, 0.50;$NaNO_3$ 2.50; K_2SO_4, 1.00; NaCl, 1.00; $MgSO_4.7H_2O$, 0.20; $CaCl_2.2H_2O$, 0.04; mineral solution, 6mL and vitamin B12, which was suggested by University of Texas Culture Collection. The culture medium was sterilized in an autoclave at 121 °C for 20 min.

2.2. Culture conditions

Arthrospira platensis was grown autotrophically on 100 mL of sterile culture media (initial pH of 6.8) in 250-mL Erlenmeyer flasks at 28 ± 2 °C, under a constant fluorescent light intensity of approximately 74 µmol photons m^{-2} s^{-1} measured by a LI-250 Light Meter with a LI-190 quantum sensor (LI-COR, USA). Agitation during cell growth was provided by shaker (TECNAL BRAND, MODEL TE-1004). Initial cell concentration was 50 mg L^{-1} for all the cultivation conditions. Each 24h samples were taken from the flasks to determine the cell density.

2.3. Determination of cell dry weight and optical density

Cell concentration (optical density) was estimated by absorbance of the suspension at 560 nm (LEDUY and THERIEN, 1977) with a UV–vis spectrophotometer. A calibration curve between optical density and cell dry weight was developed by filtering the aliquots on pre-weighed GF/C filter paper. The filtered cells were dried at 105 °C until constant weight was obtained and cooled to room temperature in desiccators before weighing. Subsequently, the culture was centrifuged at 10,000 rpm for 20 min at 4 ° C and the dry biomass used in the compounds extraction step.

2.4 pH determination

The pH was measured using a potentiometer (METTLER TOLEDO M300).

2.5 Microalgae biomass extracts preparation

Biomass extracts were prepared as described in Chu *et al*. (2006), Dinh *et al*. (2009) and Roman Bermejo *et al*. (2001) methodologies. In the first step, the cells were suspended in 200 ml of TBS buffer (Tris-HCl - saline) of pH 7.4 and containing 25 mM NaCl at 4 ° C. In the second, samples was carried out with 2 volumes of 0.02 M phosphate buffer, pH 7.0 containing 0.85 % NaCl (saline - PBS) at 4 ° C. In the third step, the suspension was frozen in 1M sodium acetate (pH 5.5) buffer. In three cases, the microalgae biomass extracts were placed under sonication in ice bath by 10 cycles of 10 minutes. Aliquots were removed and centrifuged at 5,000 rpm 7 min, 4° C, using the supernatant for hemagglutinating activity with dilutions of 1:5, 1:10 and 1:20.

2.6 Determination of hemagglutinating activity (HA)

The determination of hemagglutinating activity (HA) in the supernatants was performed in microtiter plates, following the method described by Correia and Coelho *et al*. (1995). Extracts preparation (50 µL) was two-fold serially diluted with 0.15 M NaCl. After that the addition of a 50 µL suspension of rabbit erythrocytes treated with 2.5% (v/v) glutaraldehyde was realized. After resting for 45 min, HA was expressed as the highest dilution exhibiting hemagglutination. Hemagglutination was observed macroscopically and judged as positive in the case that more than 50% of erythrocytes in the well were agglutinated. Hemagglutination activity was expressed as a titer and the reciprocal of the highest two-fold dilution exhibited positive hemagglutination. The assay was carried out in triplicate for each test solution.

3. RESULTS AND DISCUSSION

Table 1 shows the results of hemagglutinating activity of *Arthrospira platensis* biomass extracted in different buffers using sonication method. Negative hemagglutinating activity was observed in the biomass extraction by Tris-HCl-saline buffer. However, faster positive results were obtained in sodium phosphate buffer (1:10 and 1:20 dilutions) and sodium acetate buffer (1:5 and 1:10 dilutions) with high hemagglutination titers of 2^{12} (4,096) in the first buffer.

Many studies have been reported the ability of agglutination of macroalgae extracts (Chu *et al*., 2006; Fábregas *et al*. (1984,1985) ; Munoz *et al*., 1985). Phosphate buffer gained prominence in our research, for being the best buffer show hemagglutinating activity to extract from the cyanobacterium *Arthrospira platensis* using sonication method. This data corroborated studies by

LE DINH *et al*. (2009) that used the same buffer to extract agglutinins of several species of macroalgae with the range of hemagglutination between 2^2 and 2^6.

Table 1. Hemagglutinating activity of *Arthrospira platensis* biomass extracted in different buffers (sodium phosphate, sodium acetate and Tris-HCl saline)

Buffer type	Extracts dilutions	Hemagglutinating activity
Sodium phosphate	1:10, 1:20	2^2 a 2^{12}
Sodium acetate	1:5, 1:10	2^2 a 2^7
Tris-HCl saline	Pure extract,1:5, 1:10, and 1:20	Negative

In 1981, Hori *et al*. used human ABO system, erythrocytes of rabbit, horse, sheep, duck and chicken and others and found hemagglutinating activity in 14 of the 53 species tested, and the authors also observed that rabbit erythrocytes are the best suited to agglutinate compounds from algae biomass for prove more sensitive to the action of hemagglutinin present in these organisms. Similarly, FÁBREGAS *et al*. (1984,1985) and MUNOZ *et al*. (1985) working with green and red algae collected from Spain coast, observed that 100% of the tested algae were able to agglutinate rabbit erythrocytes and the agglutination was quite poor when used erythrocyte ABO system. SAMPAIO *et al*. 1993 also showed some algae the ability to agglutinate erythrocytes of rabbit preferentially. For these reasons, erythrocytes of these animals were used in our hemagglutination tests.

4. CONCLUSION

We conclude that both buffers tested were suitable for biomolecules extraction with higher hemagglutinating power against rabbit erythrocytes, highlighting the phosphate buffer as the best. Maybe, this buffer presents a pH near to neutral, and prevents the denaturation or conformational changes of biomolecules, mainly for proteins. Thus, A. *platensis* can be recognized as a potential source of new bioactive compounds of interest for biotechnological applications in the fields of human and veterinary medicine because of the possible presence of a non-immunological protein compound that binds to the blood, thus confirming the potential of these biomolecules in future studies for diagnostic medicine.

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