**Biocompatible electroextraction of proteins from microalgae: protein characterization by mass spectrometry**

Vincent BLANCKAERT, Hélène GATEAU, Justine MARCHAND, Benoît SCHOEFS\*

Metabolism, Bioengineering of Molecules from Microalgae and Application , Mer Molécules Santé, IUML – FR 3473 CNRS, Le Mans University, Le Mans, France

*\*Corresponding author: benoit.schoefs@univ-lemans.fr*

**Highlights**

* Water-soluble proteins can be electroextracted from green algae
* The electroextraction is biocompatible
* 50% of the proteins originate from the chloroplast

**1. Introduction**

The increase of human populations and the expansion of cities contribute to an accelerated depletion of agricultural lands and natural resources. To overcome these difficulties, alternative sources of molecules are searched. Among these, microalgae are promising because they produce various molecules with pharmaceutical, nutritional and cosmetic properties (Mimouni et al., 2012). Biotechnological extraction process of these molecules starts with growing biomass, then harvesting microalgae and processing according to a definite downstream program. Vinayak et al. (2015) pointed out disadvantages of these downstream processes i.e. unselective extraction of intracellular compounds, requiring additional purification step(s), waste generation but also energy and time consumption. The need to increase the durability of biotechnological processes requires the development of new extraction methods such as electroextraction. Here, the identification of biocompatibly electroextracted proteins from the green alga *Haematococcus pluvialis* is reported.

**2. Methods**

*Haematococcus pluvialis* was grown mixotrophically. Proteins were electroextracted using pulse electric fields (PEF) of 0.5 or 1 kV cm-1. After concentration and filtration, proteins were solubilized and separated by 1DE and 2DE. The resulting material was analysed by mass spectrometry: NanoLC/ESI LTQ-Orbitrap Velos ETD and MS/MS. The raw data were obtained after searching with Mascot Daemon search engine. *Chlamydomonas* was used for comparison because *H. pluvialis* belongs to the same order and it is a model organism (Marchand et al. 2018). Peptides and proteins were confirmed by generating a random peptide Bank "decoy", where the threshold of false positives has been set at 1% for peptides and proteins.

**3. Results and discussion**

The protein MW ranged from 12.4 to 164.9 kDa confirming that the proteins electroextracted are in a system without proteases. Proteins were identified by microsequencing after 1DE using nanoLC/ESI LTQ-Orbitrap Velos ETD and MS/MS. At maximum 36 proteins could be identified (1 kV cm-1) among which 16 proteins were also electroextracted at 0.5 1 kV cm-1. The localization of the identified proteins indicates that they originate from different cell compartments (Table 1). The comparison of the proteins patterns obtained with PEF of 0.5 kV cm-1 (Fig 1A) and 1 kV cm-1 (Fig 1B) when analysed by 2DE revealed that 8 spots were present only with PEF at 1 kV cm-1.

**Table 1**. Identity of the 5 top scoring electroextracted

|  |  |  |  |
| --- | --- | --- | --- |
| Uniprot/Protein | MW (kDa) | Peptide matches0.5/1 kV cm-1 | Role and location |
| Ribulose bisphosphate carboxylase large chain | 52.6 | 0/23 | CarboxylationOxidative fragmentation of the pentoseLocation: chloroplast  |
| 14-3-3 protein  | 29.3 | 0/20 | Chaperonne Location: subcellular |
| 14-3-3-like protein related | 29.5 | 0/18 | ChaperonneLocation: subcellular |
| Tubulin beta chain | 49.6 | 0/17 | Constituent of microtubulesLocation: cytoplasmic |
| ATP synthase subunit beta | 43.8 | 0/15 | [ATP synthesis coupled proton transport](https://www.ebi.ac.uk/QuickGO/term/GO%3A0015986)Location: chloroplast |



**Figure 1.** 2-DE of protein electroextracted from *Haematococcus pluvialis* PEF at (a) 0.5 kV cm-1 or (b) 1 kV cm-1.

**4. Conclusions**

PEF of 1 kV cm-1 were more efficient in protein electroextraction than 0.5 kV cm-1 from the green alga *H pluvialis*. This work confirms the possibility of using PEF technology in blue biotechnology and opens new avenues for the development of biocompatible extraction possibilities.

**References [Calibri 10]**

1. Mimouni, V., et al. (2012). "The potential of microalgae for the production of bioactive molecules of pharmaceutical interest. ." Current Pharmaceutical Biotechnology **13**: 2733-2750.
2. Vinayak, V., et al. (2015). "Diatom milking: a review and new approaches." Marine Drugs **13**(5): 2629.