Polytech network form for PhD Research Grants from the China Scholarship Council

This document describes the PhD subject and supervisor proposed by the French Polytech network of 14 university engineering schools. Please contact the PhD supervisor by email or Skype for further information regarding your application.

Supervisor information	
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University name	Université Clermont Auvergne
Country	France

PhD information	
Title	**Adaptation of the moss *Physcomitrella patens* to changes in CO_2 and temperature cultivated in bioreactor**
Main topics regards to CSC list (3 topics at maximum)	V-11. Change of world climate and climatic forecasting II-13. Green chemistry II-2. Animal and plant new transgenic techniques
Required skills in science and	Molecular Biology, cell Biology, Bioengineering,

engineering	Chemical engineering, Biosystems

Subject description (two pages maximum)

Scientific context of the PhD

According to the fifth Assessment Report released by the Intergovernmental Panel on Climate Change (http://www.ipcc.ch/report/ar5/syr/), global surface temperature change, for the end of the 21st century, is projected to exceed 2°C. The rise in the average global temperature will be associated with a higher frequency and a longer duration of heat waves on daily and seasonal timescales and a higher concentration of CO_2 in the environment linked to a constant increase of human activities. These changes are likely to have negative effects on the yield of major crops. The moss, *Physcomitrella patens,* could be used as a model plant to study the environmental effects such as CO_2 and temperature increase on specific adaptation mechanisms.

The moss, *Physcomitrella patens*, belonging to the bryophytes family, is one of the first terrestrial plants (450 million years). This anatomically rudimentary plant (leafy stem and rhizoid), *P. patens*, is very adaptable to environmental conditions and has a high biomass potential. *P. patens* can be cultivated *in vitro* during its entire cycle. The germination of the spores gives rise to a haploid vegetative filamentous tissue, which is easy to maintain in this form in a liquid medium.

The *in vitro* cell photobioreactor culture of *P. patens* is currently in full development. In particular, it is widely considered for the production of recombinant proteins and biomolecules with pharmacological activities from mutant strains (Huang et al., 2012), mainly because of the specific post-translational modifications of the plants to generate N- glycosylated mammalian-like proteins (Reutter and Reski 1996, Decker and Reski 2004, Decker and Reski 2012, Decker et al, 2014). In addition, the genetic transformation technologies of *Physcomitrella patens* have been optimized under laboratory conditions (Reski, 1998b, Reski, 1999, Schaefer, 2001) and its genome is sequenced (Rensing et al., 2008). These tools offer the possibility of creating mutants targeted for key metabolic genes of interest (primary and secondary) involved in the biosynthesis of molecules of industrial interest.

Plants, and in particular *P. patens*, have developed molecular and biochemical mechanisms of adaptation to culture conditions through a modification of their metabolisms (Reski et al., 2004). These processes enable them to synthesize molecules that can compensate the possible adverse effects of these modifications on growth and development (Nagao et al., 2005, Erxleben et al., 2012). These molecules can reach economic interests (such as for example sugars, lipids, or pigments). Although some studies have shown the possibility of cultivating *P. patens* in photobioreactor, the optimization of photobioreactor culture conditions for the production of metabolites of industrial interest remains to be developed. However, one study has shown that optimal growth conditions depend on parameters such as continuous illumination, or aeration supplemented with CO_2 (Hohe et al., 2005). This study also shows that the modification of these growth parameters in bioreactor can lead to significant morphological changes completely reorienting cell metabolism. It has also been shown that in certain culture conditions, in response to an environmental stimulus, *P. patens* is able to produce metabolites of industrial interest specific to the response to an exogenous signal (Erxeleben et al., 2012).

Positioning and objectives of the PhD

In the current context of global warming partly related to the increase of greenhouse gases, this PhD project aims at studying the potential modes of valorization of the increase of the temperature and CO_2 of the culture of the moss *P. patens* by researching metabolites of interest. In 2016, a preliminary study was initiated in collaboration between the GDEC laboratory and the Pascal Institute with a student of a Master of Science on the *P. patens* wild type in order to parameterize the growing conditions of the moss in photobioreactor. The results obtained showed that the biomass has 20 to 30% of sugars, among which, certain monosaccharides and complex polysaccharides of potential industrial interests in cosmetics or pharmaceuticals, could not be finely characterized and would require a thorough investigation.

Also, and based on the expertise acquired during the preliminary study, the PhD aims at evaluating the effect of the modification of the CO_2 level and the temperature rise in photobioreactor on the production of primary and secondary metabolites of industrial potential as well as on the modification of the expression of the associated genes.

The proposed PhD has three objectives:

- Firstly, using a metabolomic approach, the first objective will be to characterize the metabolites produced in response to 3 levels of CO_2 (high, physiological and low) and 2 temperature levels. This part of the PhD will be based on the expertise of the Pascal Institute, which has been developing for several years a similar approach for the cultivation of micro-algae.
- In a second step, by a global transcriptomic approach (RNASeq), carried out in the university team of the GDEC laboratory (Genetics, Diversity and Ecophysiology of Cereals), the second objective will be to identify the differentially expressed genes in response to the elevation of CO_2 and temperature.
- Finally, thanks to the knowledge of genes and metabolites of interest that are activated and / or inhibited in response to CO_2 modifications and to the temperature rise resulting from the first two objectives, the last objective will be to generate mutants by an approach of genetic engineering whose expression of target genes would be activated or inhibited using innovative genome editing technologies (CRISPR-CAS9) developed by the GDEC laboratory.

The candidate will use the techniques of molecular biology (cloning, PCR, RNASeq), cell biology (subcellular localization and BiFC) and functional analysis in plants (transgenesis using CRISPR-CAS9 technology, characterization of mutants ...), but also analytic techniques such as chromatography.

All the results obtained should lead to propose a physiological model gathering the metabolic pathways specifically involved in response to the elevation of CO_2 and temperature.