Exploring Ultrasounds as a tool to enhance biomass productivity and metabolite yields in evolutionary distant organisms

State of the art

Unsustainable energy and land use has unequivocally caused global warming, with nefarious environmental consequences. Rapid and far-reaching transitions across all sectors are necessary to mitigate the environmental impact and the consumption of non-renewable resources from human activities [1]. One of the more feasible and effective option is to pursue a transition to a sustainable circular bioeconomy centred on new sustainable processes. Bioprocesses are production processes that employ living cells or their components to produce desired products as an alternative to less sustainable productions [2]. Bioprocessing productivity is currently limited by low bioconversion, low productivity, and high costs of downstream processing. Tremendous research interest has focused on optimizing upstream and downstream phases to improve yields while reducing production costs [3]. Within the upstream bioprocessing it's fundamental to identify the optimal conditions in terms of medium composition, bioreactor type, cultivation mode and overall process parameters but further optimization is still needed to obtain high productivities and increased product quality [4].It has been found that *eustress* imposed on cell cultures (such as nutrient starvation, temperature, light, salinity and pH conditions) results in stimulation of biomass growth and the biosynthesis of specific compounds. Among the innovative technologies studied to induce cellular stress there are technologies based on ultrasound (US) and on pulsed electric field (PEF). Both are promising technologies to be further studied, being flexible, relatively easy to scale-up and environmental-friendly technologies; PEF thought, has a high capital cost while US has a relatively low capital cost, depending on the required energy input. USs in a liquid medium propagates as pressure waves, that can be perceived by mechano-sensing elements within the cells, inducing an intracellular response [5,6]. Different studies showed that US treatment during cultivation could affect metabolic pathways and enzymatic activity as well as improving cell membrane permeability and aeration diffusion in the culture medium promoting substrate transmembrane transportation [7,8]. US stimulation has already been tested on various industrially relevant organisms, employed in the agri-food industry, in the production of pharmaceuticals and biofuels as well as in bioremediation processes with encouraging results. Low frequency US stimulation increased the growth rate of different bacterial strains when tested on adhered cells, and in suspended cultures on *E. coli* [9,10]. *S. cerevisiae* treated with US showed an increase in biomass and cell membrane permeability evaluated through extracellular protein, nucleic acid and fructose-1,6-diphosphate contents [11]. Different strains of microalgae showed similar results from US assisted growth, obtaining enhancements in biomass and macromolecule yields, giving indication of a possible conserved mechanism for the adaptation to this eustress [6]. Macro fungi gathered interest as dietary supplements, nutraceuticals and pharmaceuticals as a promising sustainable source. US treatments on Basidiomycota promoted growth and EPS production through an increase in mycelia permeability and reduced diameter hence promoting mass transfer [12]. The application of US stimulation technology, while promising, is currently limited by the need of platforms able to dispense stable and characterized US stimulation with optimal wave frequency, power and duty cycle tailored on the specific organism and process of interest. To answer this needs, the research group I would work with developed a series of adaptable innovative devices to be integrated with existing photobioreactor and fermenters, thus creating Sono-Bioreactors (SNBR) (Ortenzi et al., in preparation).

Aims

The main aim of this research work will be to contribute to the further development of the SNBR and their integration to different cultivating platforms. Industrially relevant organisms, spanning from Bacteria to Eukarya will be selected and subjected to US treatment, as an optimization strategy to enhance biomass and macromolecules levels and to carry out an eco-physiological study on the molecular mechanism mediating mechano-sensing, the adaptation response to the mechanical stress imposed by US stimulation and its conservation in diverse (micro)organisms. The results of this work will hopefully contribute to understand the relevance of US-based technologies in the optimization of sustainable industrial bioproduction.

Methods

- Microscopy observation, fluorescent markers-assisted assays
- Optical density, Cell dry weights and Cell count analysis to monitor biomass production
- Protein by Bradford, carbohydrates by Dubois, lipids by Ehinmen for quantification
- HPLC, GC-MS for target macromolecule studies
- ConSurf Evolutionary conservation profiles of proteins
- STRING for functional protein association networks

Work Plan

1st year:

- Setting of optimal US conditions and testing of selected microorganisms for biomass and macromolecules level enhancement.
- Macromolecular quantification analysis with a focus on industrial relevant product.
- *In silico* study of mechanosensing receptors/elements (interactions and evolutionary distance studies).

2nd year:

- Setting of optimal US conditions and further testing of selected organisms for biomass and macromolecules level enhancement.
- Macromolecular quantification analysis with a focus on industrial relevant product.
- *In silico* study to include the new organisms in the previous work.

3rd year

- Scaled up testing of target organism in collaborations with companies involved in the project.
- *In silico* study of US induced cellular signalling and functional conservation of the mechanosensing apparatus.
- Results dissemination and thesis work.

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