Proteomic Profiling Reveals Differential Protein Patterns in *Galdieria sulphuraria* extracts replacing fetal bovine serum in muscle cell culture

Svenja Hütker[1], Hanna Wellkamp[1], Verena Tellström[2], Imke Lang[1], Felicitas Berger[1], Shourjo Ghose[3]

[1] Bremerhaven University of Applied Sciences, Institute EcoMaterials, An der Karlstadt 8, 27568 Bremerhaven, Germany, [2] Bruker Daltonics GmbH & Co. KG, Fahrenheitstr. 4, 28359 Bremen, Germany, [3] Bruker Scientific, LLC, 40 Manning Rd, Billerica Massachusetts 01821, United States

Learn more about 4D-Multiomics

Introduction

In recent years, environmental sustainability and ethical concerns surrounding livestock production have driven the development of cell culture–based food alternatives. However, cultivated meat production still depends on fetal bovine serum (FBS) to support cell proliferation and metabolic activity. Replacing FBS is therefore crucial for achieving more ethical, sustainable, and cost-effective production processes. This study evaluates protein-rich extracts from the red microalga *Galdieria sulphuraria* as substitutes for FBS during the cultivation of C2C12 myoblasts. While crude algal extracts inhibited cell proliferation and reduced viability, heat-treated extracts successfully replaced FBS, even during long-term cultivation. Proteomic analyses revealed distinct protein composition patterns. Ultimately, the identification of cytotoxic and growth-promoting proteins could support the optimization of algae-based, serum-free alternatives for cultivated meat production..

Methods

Crude and heat-treated extracts of *Galdieria sulphuraria* were subjected to tryptic digestion followed by peptide clean-up using the iST sample preparation kit (PreOmics, Planegg, Germany). Following resuspension, peptide concentrations of the individual samples were determined and adjusted to 500 ng/µl.

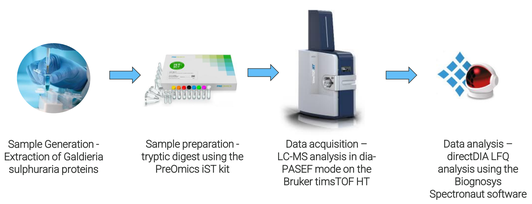

Fig. 1 Workflow description for the proteomics analysis of *Galdieria sulphuraria* crude and heat teated protein extracts.

500 ng of tryptic peptides of each sample were then injected onto a nano-LC system (nanoElute 2, Bruker Daltonics, Bremen, Germany) and eluted using a 22 min gradient. The acquisition of mass spectra data was conducted in dia-PASEF mode on a timsTOF HT mass spectrometer (Bruker Daltonics, Bremen Germany). The resulting datafiles were co-processed in a direct DIA search and evaluated in a label free quantitation approach using the software Spectronaut (BiognosysAG, Schlieren, Switzerland) (Fig.1).

Results

The effects of crude (*CE*) and heat-treated (*HE*) protein extracts from *Galdieria sulphuraria* on C2C12 myoblasts differed markedly with respect to cell growth. CE supplementation resulted in pronounced cytotoxic effects, characterized by reduced cell viability, decreased metabolic activity, altered cell morphology and impaired proliferation (Fig.2). In contrast, HE supported cell viability and metabolic activity under serum-reduced conditions and enabled gradual replacement of fetal bovine serum during long-term cultivation. HE-treated cells maintained stable growth, metabolic function, and retained their capacity for terminal myogenic differentiation.

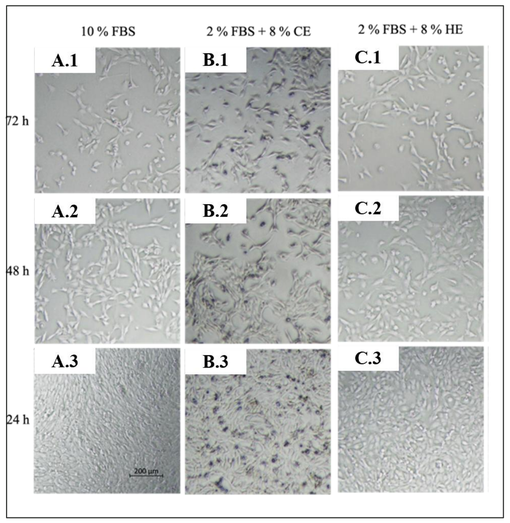

Fig. 2 Light microscopic images of C2C12 cells taken at 4 x magnification. Scale bar: 200 µm.

Comparative label-free proteomic analysis revealed clear compositional differences between CE and HE. Principal component analysis showed a robust separation of CE and HE samples, with the first principal component explaining the majority of variance, underscoring reproducible qualitative and quantitative differences in protein profiles (Fig. 3). Hierarchical clustering and heat map visualization confirmed the loss of distinct protein groups following heat treatment, consistent with heat-induced precipitation or denaturation of proteins associated with cytotoxicity. Simultaneously, specific proteins were enriched or became more prominent in HE, suggesting selective retention or functional activation of growth-promoting components (Fig. 4).

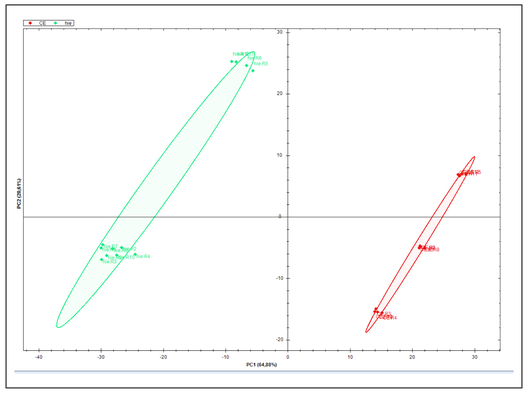

Fig. 3 Principal component analysis (PCA) of proteomic data showing clear separation between CE and heat-treated extracts (HE) along PC1.

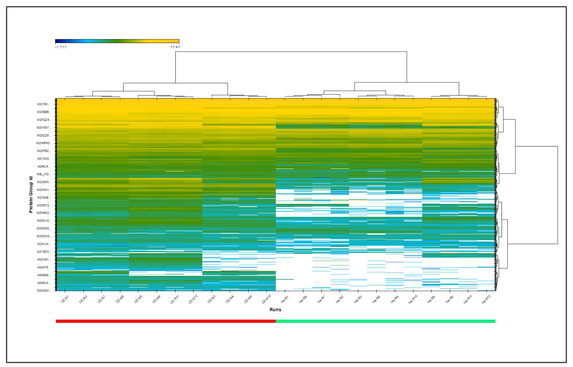

Fig. 4 Heat map of clustered proteomic profiles indicating distinct protein patterns between CE and HE, with loss or enrichment of specific proteins after heat treatment.

Summary

- *Galdieria sulphuraria* protein extracts can support long term cultivation of mammalian cell lines und FBS reduced conditions.
- Crude extracts of the microalgae express cytotoxic effects whereas heat treated extracts can be used to replace 80% of the FBS supplement (Fig. 2).
- Analysis of the protein content of *Galdieria sulphuraria* was easily accessible by combining streamlined sample preparation (iST kit), LC-MS data acquisition (timsTOF HT) and data analysis (Spectronaut) (Fig. 1).
- Statistical analysis of the proteomics data revealed clear separation in PCA analysis (Fig. 3).
- Hirarchical clustering and heat map visualization helps to identify protein groups that are either depleted via heat treated or become enriched (Fig. 4). These might represent proteins that have a cytotoxic effect on mammalian cell lines (depleted after heat treatment) or show growth promoting activities (enriched after heat treatment).

Conclusion

- Heat inactivated protein extracts of *Galdieria sulphuraria* represent a promising alternative to FBS in cell culture based meat production.
- LC-MS based characterization of the protein composition can serve as a tool to understand the growth inhibiting and promoting effects of algal extracts in order to optimize the extraction procedure.

timsTOF HT

Shourjo Ghose and Verena Tellström are employees of Bruker and may hold financial interests in the company.