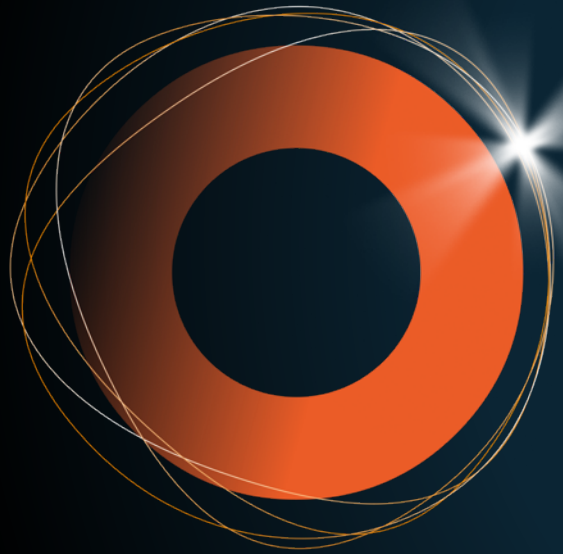

Predictive multi-omics signatures: Alternative CQ tools for biomanufacturing

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MICROBIOLOGY TECHNOLOGY INSTITUTE

To bring you **applications solutions** to identify and master key drivers **to improve bioproduction**

- Omics technology & machine learning as analytical tools
- to streamline development bioproduction
- & advanced biomanufacturing robustness

Bioproduction virtuous ladder

To identify & master key drivers to improve bioproduction

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Lab scale

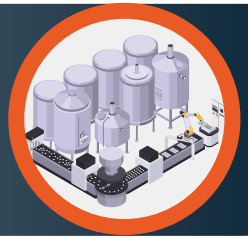
Characterize & understand biological systems, Mechanisms & physiological changes occurring in the cell

- Engineering of a genetically modified organism
- Culture condition definition

Industrial transposition

Identify metabolic bottlenecks or pathway sinks

- Optimization of culture conditions
- Identification of predictive signature
- PAT (development of advanced analytical tools)



Biomanufacturing

Process Analytical Technologies (PAT) implementation

- Real-time monitoring of biomanufacturing
- Biomanufacturing consistency assessment

Bioproduction expected out-comes technologies

Biomass increase by reliably improve microbial fitness

Productivity enhancement (quality & quantity)
with genetically modified micro-organism
or adapting cultivation medium

Inter-batch consistency improvement
with tailored monitoring

Real-time monitoring of biomanufacturing
to allow controlled continuous cultivation

STUDY CASES

Metabolomic data to improve vaccine production

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Challenge

During viral vaccine bioprocess, accumulation of glutamate in Vero cell culture medium can be detrimental for production yield.

Study Vero cell metabolism to explain why there is a high production/accumulation of glutamate, in particular upon infection with virus

Solutions

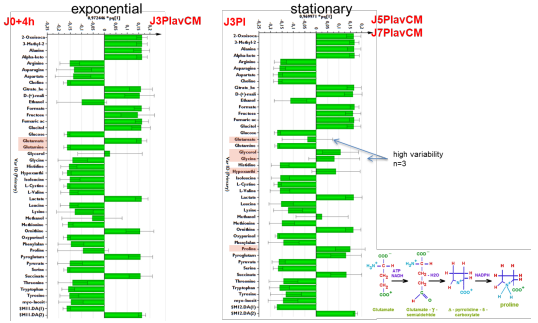
Untargeted metabolomics allow to analyze Vero cells metabolism during a 5 days propagation and infection process.

Intracellular non-polar metabolites are identified by mass spectrometry (MS).

Intracellular and extracellular polar metabolites are identified and quantified by nuclear magnetic resonance (NMR).

Results

- Glutamate is produced from Glutamine by glutaminolysis but alternatively could be produced as a side product though asparagine biosynthesis especially in the exponential phase
- Glutamate could be uptaken from extracellular media during the stationary phase to fulfill its role in malate-aspartate shuttle
- Glutamate could be converted to proline in the stationary phase
- The presence of Pyroglutamate is due to heat conversion of the Glutamine



Outlook

Metabolic data from viral production process allow a better knowledge of Vero cells metabolism. Metabolic mechanism understanding is a key to improve :

- media formulation (Amino-acids supplementations…)
- process conditions (time of infection, temperature…)

Strain engineering for antigens lipidation

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Challenge

Genetically modification of *E.coli* strain to improve :

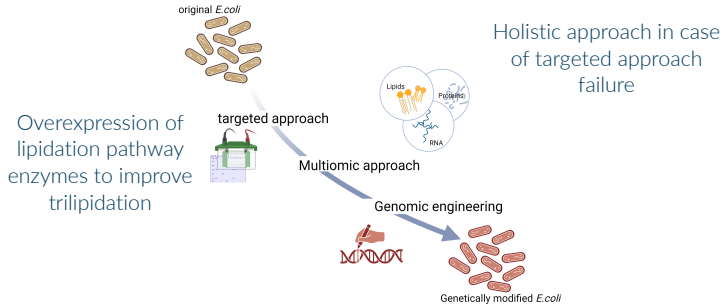
- Production yields of recombinant vaccine antigen
- Lipidation (post-translational modification) of recombinant antigen

Solutions

Identification of target for strain modification using a two-step strategy (derisking):

- Targeted approach focusing on lipidation mechanism in E.coli
- Multi-omic approach to understand metabolic pathways linked to lipidation performance

Strain modification according to identified targets



Results

- Qualitative & quantitative characterization methods of lipoprotein lipidation
- Know–how on key parameters that influence lipoprotein production

- Targeted approach did not led to significant improvement
- Transcriptomic and proteomic data obtained during bioprocess allow to identify genes to be targeted to generate genetically modified strain

Outlook

- Metabolic engineered strain for yield increase & improved robustness
- Proprietary genetically modified strain for lipoprotein production

Real-time monitoring to improve bioproduction processes

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Challenge

Monitor dynamically, and in real-time, cells in fermentation systems to understand reactions, anticipate potential issues in production & optimize processes

Solutions

- Culture of *Saccharomyces cerevisiae* yeast & continuous 1H NMR analysis for 6 hours, focusing on metabolic pathways common to all eukaryotic cells
- Metabolites monitoring in biological processes, the culture could flow from the bioreactor to the NMR spectrometer, in a closed circuit, allowing temperature stability using InsightMR™

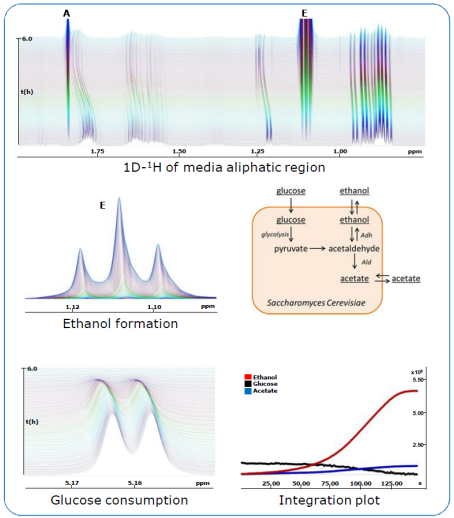
Results

In-line characterization of metabolic activity of *Saccharomyces cerevisiae*, using InsightMR™ software.

All amino acids, glucose, myo-Inositol and choline detected while vitamins, at submicromolar concentration, were under the detection limit

During the fermentation, glucose was consumed, while acetate and ethanol were abundantly produced.

Monitoring of living cells, offering a new tool for the dynamic monitoring of bioprocesses.



Outlook

NMR metabolomics data from bioprocess allow identification of metabolic pathways or biomarkers that give information about process progress. Process Analytical Technologies (PAT) can then be implemented using these biomarkers.

Antigen production consistency thanks to predictive signatures

Challenge

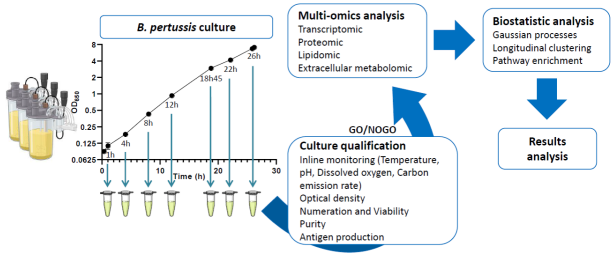
Demonstrate the potential of a multiomic approach for the identification of predictive signatures of *Bordetella Pertussis* fermentation, linked to antigen production consistency

Solutions

Fermentation process longitudinally sampled for omic analysis

Differential analysis allow to understand physiological mechanisms and metabolic pathways involved during fermentation process.

Statistical analysis can reveal signatures that are predictive of the final antigen production yields.



Results

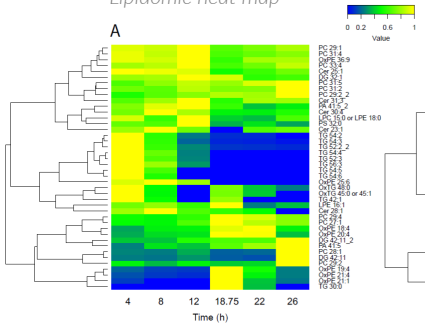
Growth slows down after 19h cultivation

- Lipidomics suggests oxydative stress & energy starvation
- Metabolomics indicates PolyHydroxyButyrate degradation, suggesting energy stock consumption

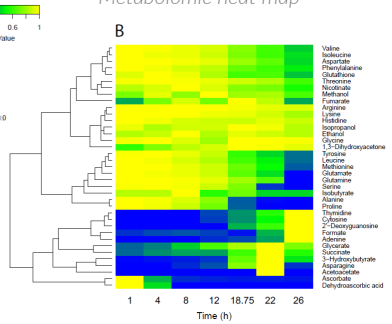
Proline starvation between 19h &22h, with still 42% of initial [glutamate] at 22h

- Decrease of proline catabolism after 12h & proline up take after 19h
- Glutamate up take increases after 19h
- Loss of reducing power & energy

Lipidomic heat map



Metabolomic heat map



Outlook

Metabolic pathways identified can be used for:

- Medium composition improvement
- Fed-batch strategy adjustment
- Identification of reasons of process variations

Molecular signatures that are predictive of batch failures could be identified. Such predictive signature may also contribute to vaccine manufacturing robustness and consistency

To bring you
applications solutions
to identify and master
key drivers
to improve bioproduction

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Bioproduction current challenges:

- To identify & master key drivers of bioproduction from lab scale, industrial transposition to biomanufacturing

Omics technologies & advanced bioinformatic pipelines:

- To characterize and understand biological systems, mechanism and physiological changes occurring in the cell

Bioproduction expected out-comes:

- Biomass increase
- Productivity enhancement (quality & quantity)
- Inter-batch consistency improvement
- Follow-up & adaptation of culture conditions by real-time monitoring of biomanufacturing

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