New facilities for bioprocess development of bioactive secondary metabolites at the HZI

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Introduction

The biotechnology unit of the Helmholtz Centre for Infection Research has recently moved to new facilities. We are involved in the production of multi-gram scale amounts of candidate natural compounds from bacteria and fungi for late stage preclinical trials that can be used to combat multi-resistant pathogens as novel anti-infective drugs. Many of these molecules are derived from hitherto unexplored sources like Myxobacteria and Basidiomycota, which are in our focus with regard to the discovery of novel anti-infective natural products. This poster is dedicated to illustrate the capabilities of our new biotechnological production plant, which is rather unique in German and European academia as both the production and the downstream processing of low-molecular, medium polar „druggable“ natural molecules can be obtained in a sustainable fashion.

LUCULLUS

Shake flask cultivations

- Respiratory Activity Monitoring System (RAMOS, Hitec Zang GmbH) equipped with sixteen flasks
- Successful transfer from shake flask conditions into the bioreactor
- Inter alia used for media development

Multifermenter system

- DASGIP (Eppendorf)
- Eight glass vessels can be operated in parallel
- 1.5 L working volume
- pH and DO control
- Punctual and continuous substrate feeding
- Process development in laboratory scale

Large lab scale

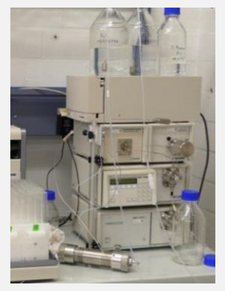
- Stainless steel bioreactors (Bbi biotech)
- 10 L working volume
- Six vessels available
- One duplex steel fermenter available for the cultivation of halophilic organisms
- Feeding profiles applicable
- Process implementation and optimization in technical scale

Pilot scale

- Stainless steel bioreactors (Heinrich Frings GmbH & Co. KG)
- Four fermenters with 150 L working volume
- Two reactors with 350 L working volume
- One fermenter each scale with duplex steel to handle halophilic processes
- Fermenter supply scientists with material for further studies
- Data can be used for process transfer to CRO’s as feasibility study

Global process characterization and organization

- Substrate and product analysis *via* HPLC-DAD/RI/MS and bio-chemical assays for full bioprocess characterization
- Additional analyses may be established
- Equipment interconnected by the global LIMPS/PIMS System Lucullus (Securecell) to compare processes of different scale and ease process scale-up and optimization.

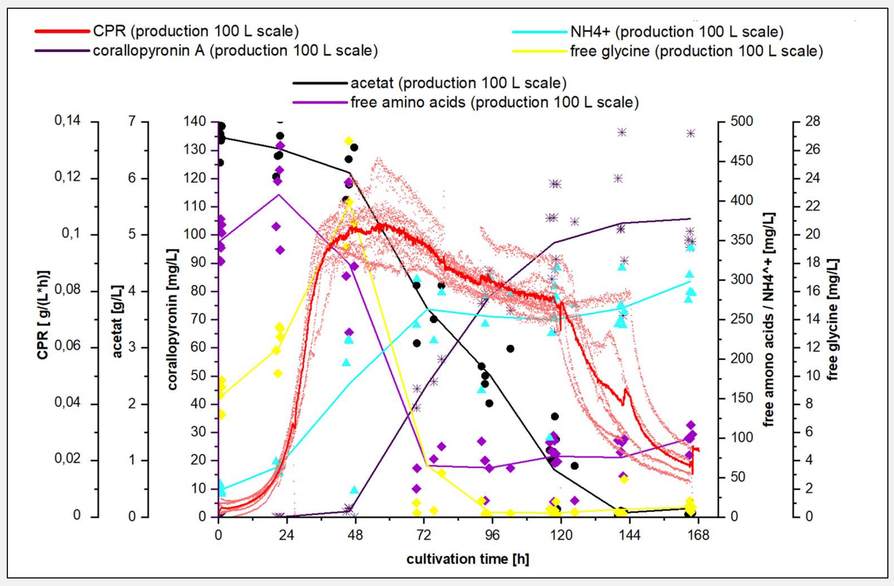


Downstream processing

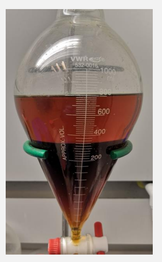
- Batch wise purification of bioprocesses in pilot scale
- Explosion protected area to handle large volumes of solvent
- Available large scale techniques:
  - Biomass separation
  - Filtration
  - Liquid-liquid separation
  - Concentration
  - MPLC
  - Preparative HPLC

Example: Coralloryronin A

- Corallopyronin A a promising preclinical antiparasitic drug candidate
- [1] with promising activity and lack of toxicity in vivo [2, 3]
- „First in class“ production by a heterologous myxobacterial host



- Recombinant *Myxococcus xanthus* DK1622 as production strain [4]
- Development of a stable bioprocess
- Comparable online and offline data of multiole repeats indicate a robust and reproducible bioprocess
- Product yields of 100 mg/L Corallopyronin A achieved



- In house purification of material from pilot scale fermenters
- Five purification steps
- 92 - 96 % product purity (^{1}H-NMR) [1]

References

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[2] K. Pfarr et al. (2018): Am J Trop. Med. Hyg. 2018; 99(4_suppl):667–8.
[3] A. K. Krome, et al. (2020): Pharmaceutics. 12(11):1105
[4] D. Pogorevc, et al. (2019): Metab. Eng. 55:201–11.

Acknowledgements

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