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Metagenomic screen for the identification of novel industrially relevant sulfatases

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libragen

biocatalysis, metagenomics & expertise

Part of Induchem companies, libragen explores and valorizes the microbial and biotransformation-based diversity: enzyme discovery, conception and development of biocatalytic processes.

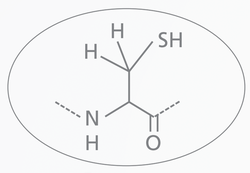
Part of the MICALIS Institute (human nutrition), Dr. Olivier Berteau's team is dedicated to the study of bacteria which inhabit the human digestive tract in order to gain insights into their function.

Objective

- Sulfatases catalyze the hydrolysis of sulfate groups of a broad diversity of substrates
- Sulfate groups have been shown to be critical for the biological properties of natural (GAGs) and synthetic sulfate-containing products

To identify performant sulfatases to be used in chemoenzymatic synthetic routes throught the exhaustive screening of bacterial genomes: metagenomic approach enabling to accelerate and to widen the discovery process.

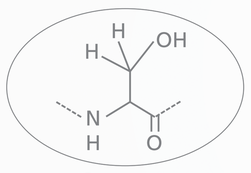
The sulfatase challenge



Cys-type

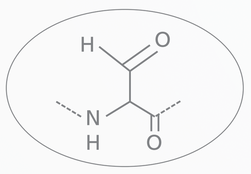
Consensus sequence (C or S)xPxR

Post-translational maturation



Ser-type

Anaerobic Sulfatase Maturing-Enzyme



Berteau *et al.*, J Biol Chem. 281: 22464-70; 2006

Strategy

Discovery of metagenomic sulfatases using a functional screening

- Ability to detect the target activity
- Having selected an expression system (host and vector), ability to express a model gene / to detect the target activity in HTS conditions
- Construction of metagenomic library
- Application of the screening to metagenomic clones
- Validation of hits and enzyme characterization

Achievements

Sulfatase detection

- Available (commercial) sulfatase (*A. aerogenes*)
- 4-Methylumbelliferyl Sulfate prefered to 4-Nitrophenyl Sulfate: fluorescence (350-460 nm) vs UV (405 nm); weak pH dependance

Expression system and method set up

- EC100™ (*E. coli*) and pEpiFOS™-5, epicentre® – 40 kbp
- Model Sulfatase: *Clostridium perfringens*
  - Sulfatase gene from *C. perfringens* ATCC 13124 inserted in pRSF (Kan-); cloning in EC100™
  - Sulfatase activity is detected only in highly concentrated cultures (e.g 275 g wet/L, 24 h)

  EC100™ is able to produce active sulfatase
  - *C. perfringens* genomic library (EC100™, pEpiFOS™-5)
  - 1152 clones ; detection of ß-Galactosidase (3%)

  C. perfringens sulfatase difficult to express and/or to maturate (consensus: CiAsR)
- Model Sulfatase: *Pseudomonas aeruginosa* (ATCC 27853)
  - Consensus: CxPxR
  - *Pseudomonas aeruginosa* genomic library (EC100™, pEpiFOS™-5)
  - 1152 clones; sulfatase activity easily detected

  P. aeruginosa sulfatase: expression and maturation in the expression system (*E. coli* and fosmid) - 96-well µplates, LB medium, 24 h growth, 4-MUF-S addition

Metagenomic libraries

Human feces (16128 clones, EC100™, 40 kbp)

Metagenomic sulfatases

- 17 positive clones (rate: 10^{-3})
- Validation (10 vs 17), sub-cloning (pBSKS, 3 kbp, EC100™), characterization
- Enzyme production and biochemical characterization

In conclusion

- Step by step procedure to identify / solve bottlenecks
- 1st example of sulfatases / metagenomic approach
- Perspectives for biotransformation processes

To get more information on this project:
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PolyModE

SEVENTH FRAMEWORK PROGRAMME