Development of improved cellulase variants for Spent Mushroom Substrate conversion by directed evolution

Cloning and recombinant expression of two cellulases produced by *Streptomyces argeteolus* AE58P in *Escherichia coli*

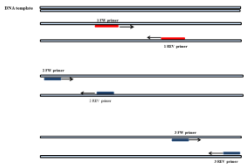
Actinobacteria strains

1

Cel1

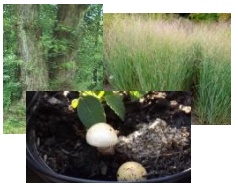
Cel2

2

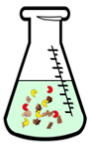


3

Selection of the best enzyme for the creation of the directed evolution library



Biomasses



4

Sugar

5

HPLC

6

Cel2

Creation and screening of the directed evolution library

EP-PCR

***E. coli* transformation**

Inoculum in 200 uL
Incubation o.n. at 28°C

A **SOLID-LIQUID Picking**

LIQUID-SOLID Gridding

B

Screening on solid medium in Qtray
Incubation for 4h at 37°C

LIQUID-LIQUID Re-arraying

C

STD-well

DEEP-well

Positives clones inoculated into 2 new multiwells for growth
-DEEP-well (1-2) filling with 900 uL
-Inoculum: 100 uL from DEEP- well into DEEP-well 1-2

DEEP-well

Addition of glycerol
STAB

D

1 DEEP-well

2 DEEP-well

GROWTH

E **CENTRIFUGATION**

F **ACTIVITY ASSAY**

Characterization of the selected evolved variants and their use for enzymatic hydrolysis

Improved Cel2 variants

Cloning and recombinant expression of two cellulases (Cel1 and Cel2) produced by *Streptomyces argeteolus* AE58P in *Escherichia coli;* characterisation of Cel1 and Cel2 and test of the enzymes on lignocellulosic biomasses to select the best enzyme (Cel2) for the construction and screening of the 30000 mutants directed evolution library to obtain evolved Cel2 variants for conversion of spent mushroom substrate

Legend

1- Selection of two cellulases produced by *Streptomyces argeteolus* AE58P, the highest cellulase activity producer among 24 Actinobacteria strains identified and screened for cellulase activity in the frame of another project, BioPoliS.

2- Cloning and recombinant expression of the new cellulases Cel1 and Cel2

3- Incubation of Cel1 and Cel2 with lignocellulosic biomasses to select the best enzyme for the construction and screening of the 30000 mutants directed evolution library

4- Hydrolysis of lignocellulosic biomasses

5- Detection of the sugars obtained after hydrolysis step by HPLC

6- Selection of Cel2 for the construction and screening of the directed evolution library