A HIGH THROUGHPUT PROFILING METHOD FOR CUTINOLYTIC ESTERASES

Pasi Halonen ● Johanna Buchert ● Tapani Reinikainen

INTRODUCTION

Cutinases are esterases that can degrade the protective surface polymer (cutin) of aerial parts of plants [1]. They are secreted mostly by phytopathogenic fungi [2, 3], and the major cutinase from Fusarium solani f. sp. pisi has been thoroughly characterized [4]. Cutinases have been shown to be promising catalysts in a variety of chemical and biotechnological applications due to their ability to operate both in aqueous and nonaqueous environments [5]. Here we describe a novel method for quick detection of cutinolytic esterases for potential utilization.

PLATE SCREENS

The primary screening of cutinolytic esterases from 38 micro-organisms was done on agar plates. Cutin, or a cutin analog, polycaprolactone (PCL), were supplied as the sole carbon source to follow microbial growth and esterase activity. Cutin hydrolysis was monitored via a pH change as a proton is released into the medium during an ester bond hydrolysis (Figure 1). Cutinase is also a PCL depolymerase [6], and a clear halo could be observed upon polymer degradation on PCL plates (Figure 1).

ROBOTIC SCREENS

The automated activity assay method was optimised for a 96-well microtiter plate format allowing high throughput. A common procedure for fast activity screening on ORCA (Optimized Robot for Chemical Analysis) was developed (Figure 2).

ESTEROLYTIC FINGERPRINTING

p-Nitrophenyl (pNP) fatty acids (carbon chain 2 – 16 atoms) were used in robotic screening to explore substrate and pH dependence of enzymes secreted in liquid media by selected micro-organisms. pH dependent isoenzymes were observed at different stages of cultivation, as the action by neutral esterases induced the production of alkaline esterases (Figure 3).

CONCLUSIONS

- A novel procedure for profiling and identification of cutinolytic esterases was developed by combining plate screens with a robotic workstation:
  1. microbial growth and extracellular esterase activity are first assessed
  2. an automated activity assay in a 96-well MTP format is used for esterolytic fingerprinting of different stages of microbial cultivation
  3. H-cutin and fluorescence microscopy are used for hit verification
- The induction of esterase isomers, and their substrate specificity and pH dependence can be monitored and quantitatively determined
- Eventually, novel cutinolytic activities with desired properties can be found

REFERENCES