CONSTRUCTION OF A NEW PRODUCING STRAIN *PENICILLIUM CANESCENS* FOR CO-EXPRESSION OF RECOMBINANT XYLANASE AND LACCASE

Koroleva O.V., Chulkin A.M., Vavilova E.A., Fedorova T.V., Benevolensky S.V.
A.N. Bach Institute of Biochemistry Russian Academy of Sciences, 117091, Moscow
E-mail: koroleva@inbi.ras.ru

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Introduction. The screening and design of the biocatalysts as well as their usage in different industrial application is the main trends of modern biotechnology. Recently, the intensive research is being conducted to develop a comprehensive strategy for the application of complex enzymes preparations in the pulp and paper industry for the bleaching of Kraft pulp, which is traditionally carried out using chlorine containing compounds. The xylanase and laccase preparations are mostly used for delignification and bleaching of Kraft pulp. The aim of the present study is construction of recipient strain *Penicillium canescens* with eliminated catabolic repression and co expression of genes encoding laccase and xylanase in this strain.

Methods. The strain *Penicillium canescens creA*$^-$ (PCA-10/I-7) possessing mutation on catabolic repression was created as described in [1]. Protocols for mutagenesis and transformation of *P.canescens* were described *ibid*. The identification of extracellular laccase and xylanase has been carried out by enzymatic analysis [2], mass-spectrometry and Western blotting using polyclonal antibodies.The cultivation of recipient strains was carried out according to [3].

Results. The strain *Penicillium canescens creA*$^-$ (PCA-10/I-7) is notable for L-arabinose dependent induction of transcription of highly expressed genes coding for endoxylanase *(xylA)* and β-galactosidase *(bgaS)*. UV-inducible mutant (PCA-10-4/I-7/12) was selected demonstrating arabinose independent pattern of *xylA* and *bgaS* genes expression. This strain is a promising host for construction on its basis different kind of enzyme producing strains. To perform further transformation experiments mutation in nitrate reductase gene (*niaD*$^-$) was selected in this strain resulting in final recipient strain PCA-10-4/I-7/12*niaD*$^-$.
Expression plasmids were constructed containing *P.canescens* endoxylanase gene *xylD* and *Trametes hirsuta* laccase gene *lac* under control of *bgaS* promoter. The co-transformation of these plasmids in the recipient strain *Penicillium canescens* PCA-10-4/I-7/12*niaD*$^-$ genome provided the obtaining of strain-producer secreting into cultural broth simultaneously both laccase and xylanase (Fig.1). By RT-PCR transformant clones were selected containing multiple copies of target genes. Production of both enzymes is independent of arabinose addition to culture medium.
The optimization of cultivation conditions of strain-producer *P. canescens* producing both recombinant laccase and xylanase has been performed. The crude enzyme preparation contained recombinant xylanase (450 U/ml) and recombinant laccase (2,5 U/ml). The optimal pH and temperature ranges for the target recombinant enzymes – laccase (pH 5,0-6,5; 40-50°C) and xylanase (pH 4,0-6,0; 40-70°C) being a part of the complex enzymes preparation has been determined.

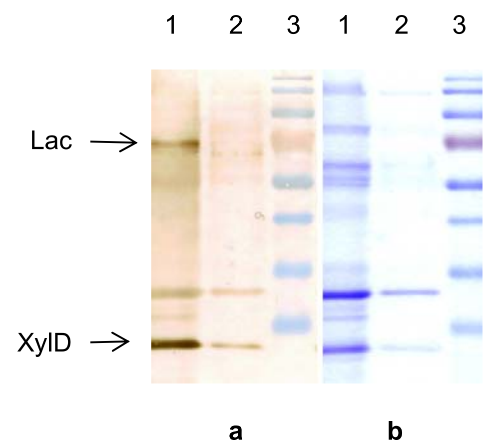

Fig.1. Western blot (a) and SDS-PAAG electrophoresis (b) of *P.canescens* PCA-10-4/I-7/12*niaD*$^-$ cultural broth. Lanes 1, 2 – cultural broth (10, 5 ng respectively); lanes 3 – molecular weight marker.

Conclusions. The recipient strain *Penicillium canescens creA*$^-$(PCA-10-4/I-7/12) demonstrating arabinose independent pattern of *xylA* and *bgaS* genes expression has been constructed. This strain could be used as a host for construction producers of recombinant enzymes.
Based on the strain *Penicillium canescens creA*$^-$(PCA-10-4/I-7/12) as a host the new producer secreting extracellular recombinant laccase and xylanase has been obtained.

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References

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