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Biocatalytic transformation of the progesterone by the *Aspergillus nidulans* VKPM F-1069 and by the genetically modified strains obtained on its basis

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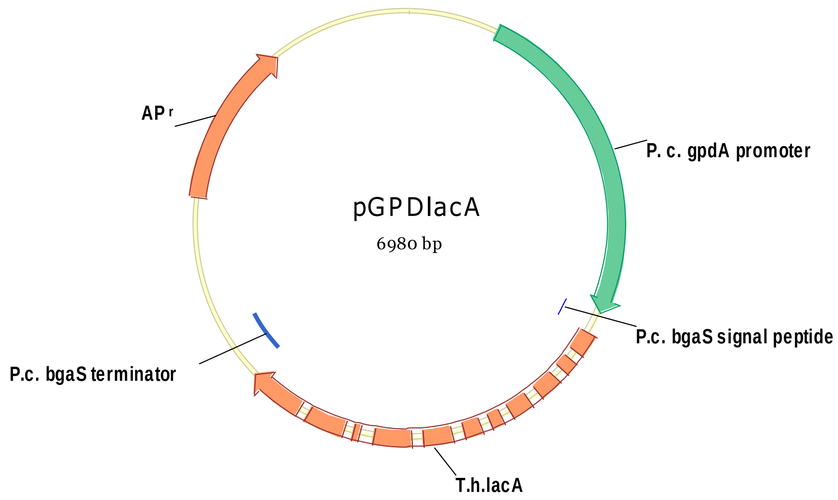
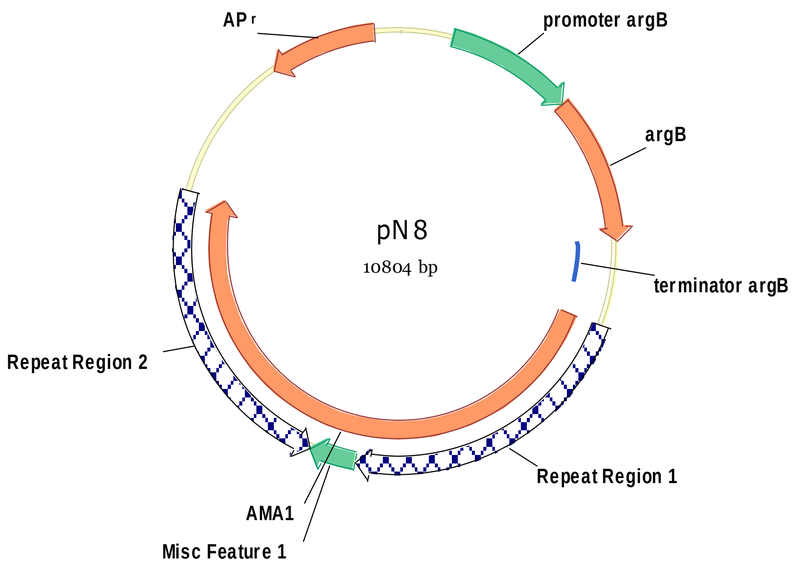
Characteristics of the used strains:

1. ***A. nidulans* VKPM F-1069** (wild type)
2. ***A. nidulans* 031 (argB$^-$; pyrG$^-$)** (syn. FP-308.1, AN031; CBS 129193) - auxotrophic strain of *A. nidulans* VKPM F-1069, with the mutation argB2 and pyrG89. The presence of the argB2 mutation leads to the inability of the strain to carry out arginine biosynthesis independently, and the presence of the mutation pyrG89 – to the inability of the strain to provide uridine and uracil biosynthesis.
3. ***A. nidulans* lac№4 (argB$^-$)** was obtained by co-transformation of the integrative plasmids pGPDlacA and pJR15 [Oakley, 1987] into the strain *A. nidulans* 031 (argB$^-$; pyrG$^-$). It is a producer of heterologous secreted laccase A of *Trametes hirsuta* 072 - a lignolytic enzyme (EC 1.10.3.2).

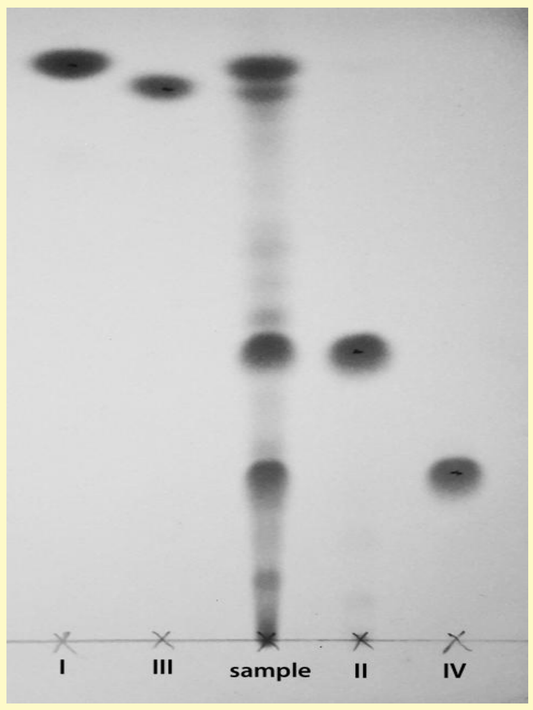


The integrative plasmid pGPDlacA: T.h.*lacA* – laccase A encoding sequence, AP^r- ampicillin resistance gene, P.c.*gpdA* promoter – promoter of the gene encoding glyceraldehyde-3-phosphate dehydrogenase of *Penicillium canescens*, P.c. *bgaS* signal peptide – β-galactosidase signal peptide of *P. canescens*, *P*.c. *bgaS* terminator – β-galactosidase transcription terminator of *P. canescens*

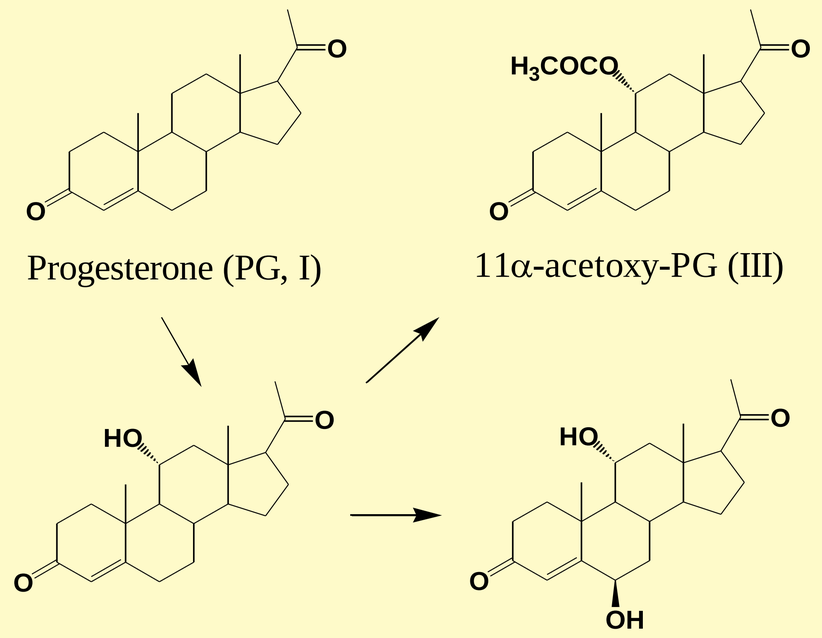
4. ***A. nidulans* 031/pN8 (pyrG$^-$)** strain was obtained by the transformation of the autonomously replicable plasmid pN8 (syn. pDHG25-SgrDI) into the strain *A. nidulans* 031 (argB$^-$; pyrG$^-$).



The autonomously replicable plasmid pN8: AP^r- ampicillin resistance gene, AMA1 - region providing autonomous replication in *A.nidulans*, argB – *argB* gene of *A.nidulans*, promoter argB – promoter of *argB* gene *A.nidulans*, terminator argB – *argB* gene transcription terminator



The TLC of extract, obtained after PG biotransformation

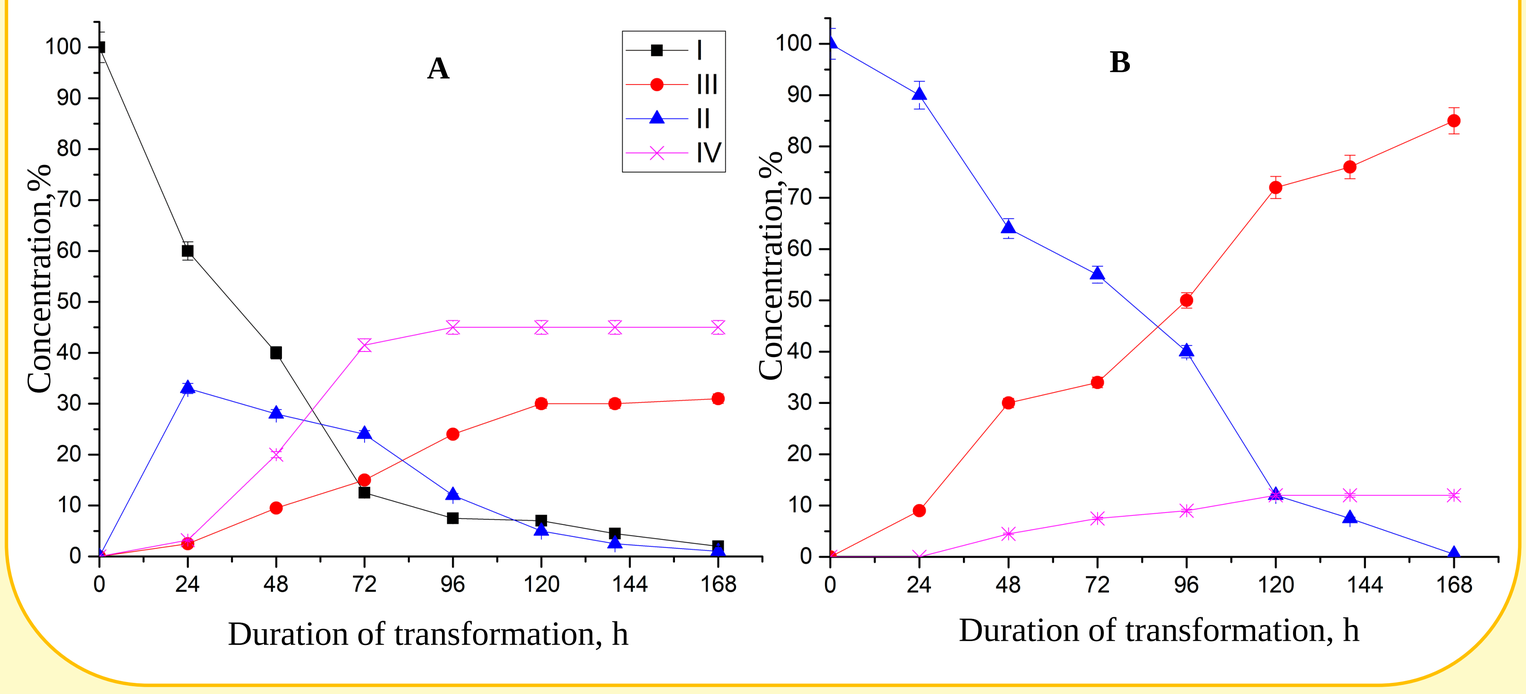


Progesterone (PG, I) — 11α-acetoxy-PG (III)

11α-hydroxy-PG (II) — 6β,11α-dihydroxy-PG (IV)

Products formed after PG transformation by *A. nidulans* strains

The kinetics of products accumulation during the PG (A) and 11α-hydroxy-PG (B) biotransformation by *A. nidulans* VKPM F-1069



The conversion of PG by *A. nidulans* strains and their 11α-monooxygenase activity

Strain	*A. nidulans* VKPM F-1069	*A. nidulans* 031 (argB$^-$; pyrG$^-$)	*A. nidulans* 031/pN8 (pyrG$^-$)	*A. nidulans* lac№4 (argB$^-$)
PG conversion , %	85,39	80,52	94,09	94,37
11α-monooxygenase activity, mol %	80,12	99,4	90,19	90,62

Relative selectivity (mol%) of the formation of PG transformation products

Strain	*A. nidulans* VKPM F-1069	*A. nidulans* 031 (argB$^-$; pyrG$^-$)	*A. nidulans* 031/pN8 (pyrG$^-$)	*A. nidulans* lac№4 (argB$^-$)
11α-acetoxy-PG	29,53	47,4	31,36	41,87
11α-hydroxy-PG	17,05	13,8	23,6	26,51
6β,11α-dihydroxy-PG	53,42	38,8	45,04	31,62

✓The formation of products was confirmed by the ^{1}H NMR, ^{13}C NMR, 2D NMR and HPLC-HRMS.

✓ At the first stage of biotransformation, PG is hydroxylated at C11α position. However the resulting 11α-hydroxy-PG is not the final product. It is further modified into 11α-acetoxy-PG and 6β,11α-dihydroxy-PG. Thus, the acetylated product can be obtained by using both PG and 11α-hydroxy-PG as the initial substrate.

✓The presence of mutations in the *A. nidulans* 031 (argB$^-$; pyrG$^-$) strain has a negative effect on the rate of the substrate conversion (compared to the wild type strain).

✓The introduction of different plasmids into the *A. nidulans* 031 (argB$^-$; pyrG$^-$) strain leads to almost an identical increase in the rate of PG conversion compared to the wild type strain. An increase in 11α-monooxygenase activity is also observed.

✓Significant accumulation of 11α-acetoxy-PG occurs during the PG transformation by the strains *A. nidulans* 031 (argB$^-$; pyrG$^-$) and *A. nidulans* lac№4 (argB$^-$). Probably, this effect may be due to the inability of these strains to synthesize arginine independently.

✓*A. nidulans* lac №4 (argB$^-$) strain is the most promising for further study, as it has the highest total relative selectivity of target compounds II and III formation and the lowest selectivity of compound IV formation.

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