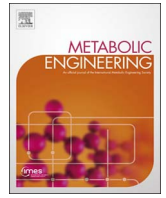


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Keywords:

Saccharomyces cerevisiae in vitro k_{cat} K_m

1. Introduction

S. cerevisiae

ff

S. cerevisiae
fi

ffi

ff

fi -
fi

ff

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E-mail address:

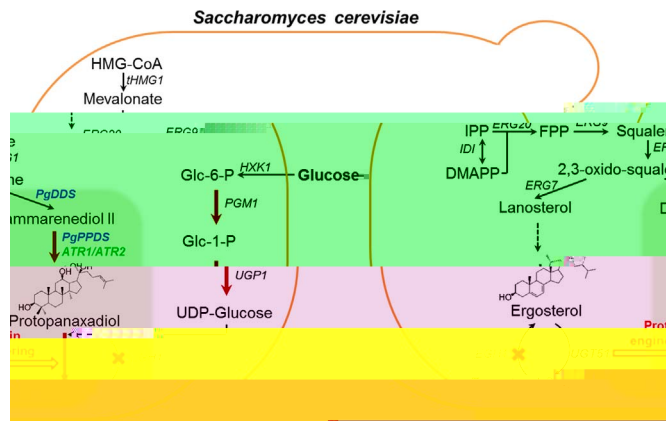


Fig. 1.

P. ginseng *A. niger*

in vitro

S. cerevisiae

S. cerevisiae

2. Materials and methods

2.1. Chemical, strain and culture condition

E. coli DE3

β

fl

fi

2.2. Production and purification of glucanase UGT51

S. cerevisiae

NdeI *XhoI*

E. coli

μ

g

ff

ff

ff

2.3. Glucanase assay

μ

ff

fi

n

fl

μ

ff

fi

n

fi

2.4. Chemical analysis

2.5. Site-directed mutagenesis and sequence alignment

2.6. Metabolite analysis

3. Results

3.1. UGT51 from *S. cerevisiae* as a promiscuous glucosyltransferase for Rh2 synthesis

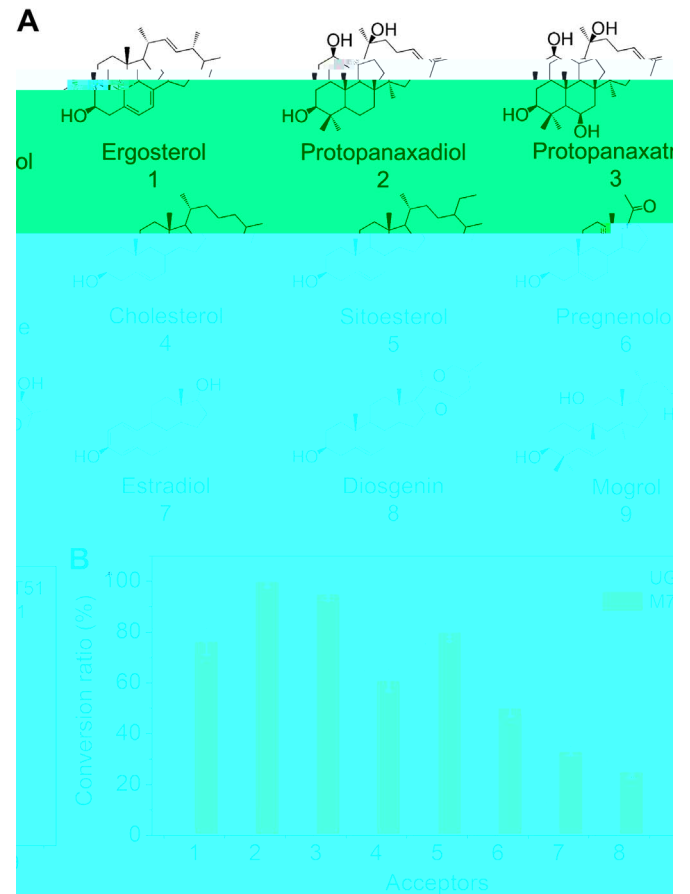


Fig. 2.

3.2. Semi-rational design of UGT51 toward an efficient Rh2 synthesis

E. coli DE3

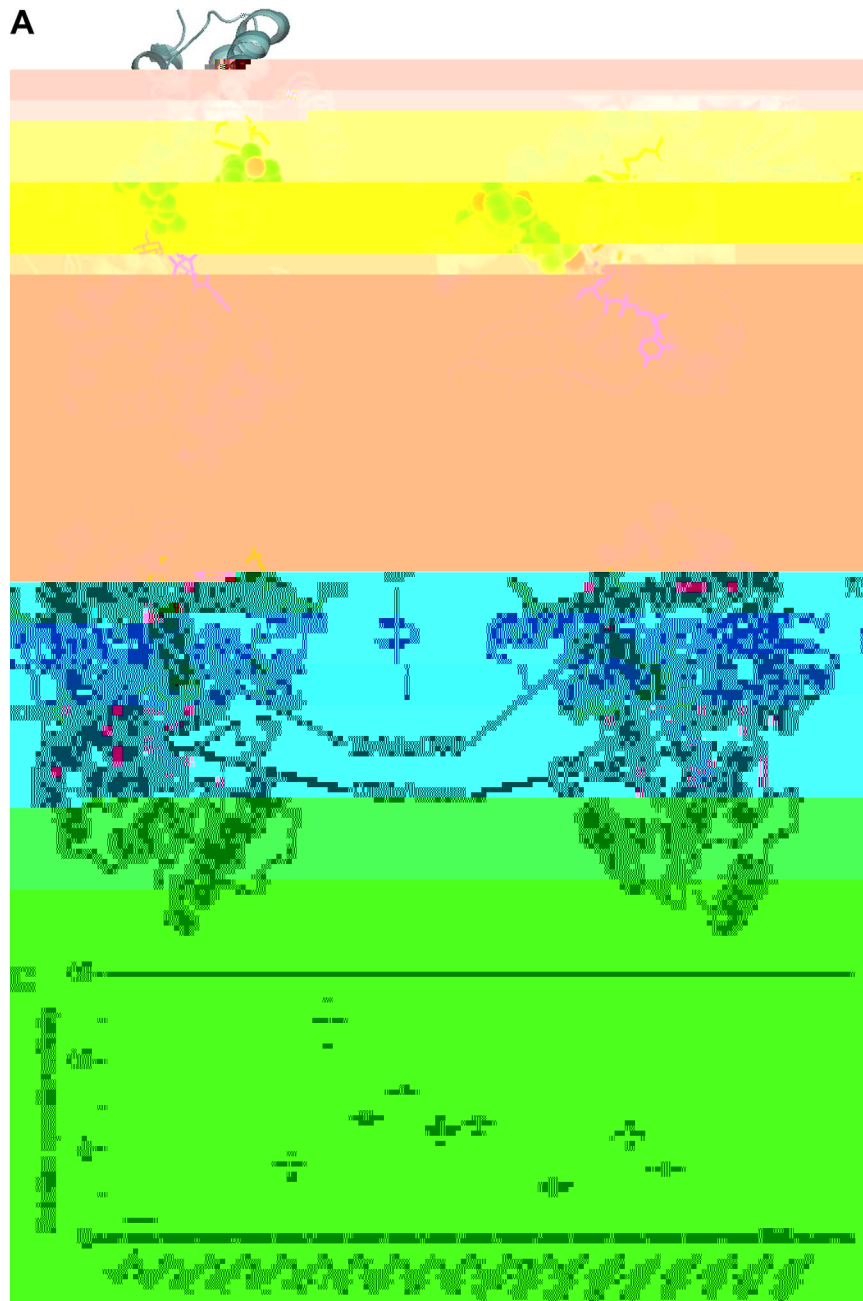


Fig. 3.

fi

fi

fi
ffi

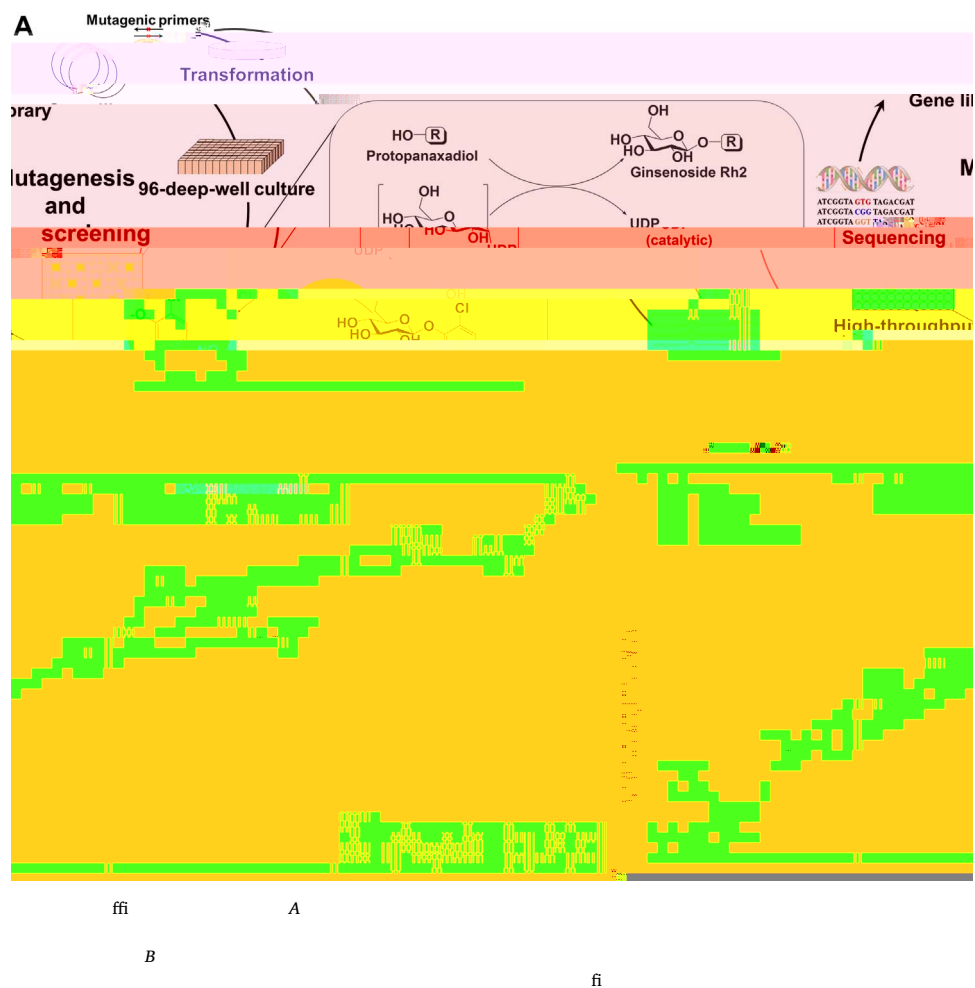


Fig. 4.

Table 1
Kinetic parameters of UGT51 and its mutants

Enzyme	Mutations	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($mM^{-1}\cdot s^{-1}$)	Fold change over WT
	-	-	-	-	-
		-	-	-	-

fi

ffi

in i ro

fi

fi

K_m k_{cat}/K_m

ffi

Δ

fi

k_{cq}/K_m

Δ

fi

3.3. Production of Rh2 in *S. cerevisiae*

S. cerevisiae

TDH3

$P_{TDH3-M7-1-T_{CYC1}}$

HO

4. Discussion

in vitro

fi

fi

ffi

ffi

fi *S. cerevisiae*

Cruciform neoforman

β

β

in vitro

EGH1
Δ

fi

Δ

Δ

HXK1, PGM1 UGP1

PGM1

PGM1 UGP1

PGK1
Δ HXT7

Δ

PgPPDS

ffi

ffi
PPDS-75 ATR2

ffi

ffi

Δ

δ
Δ

Δ

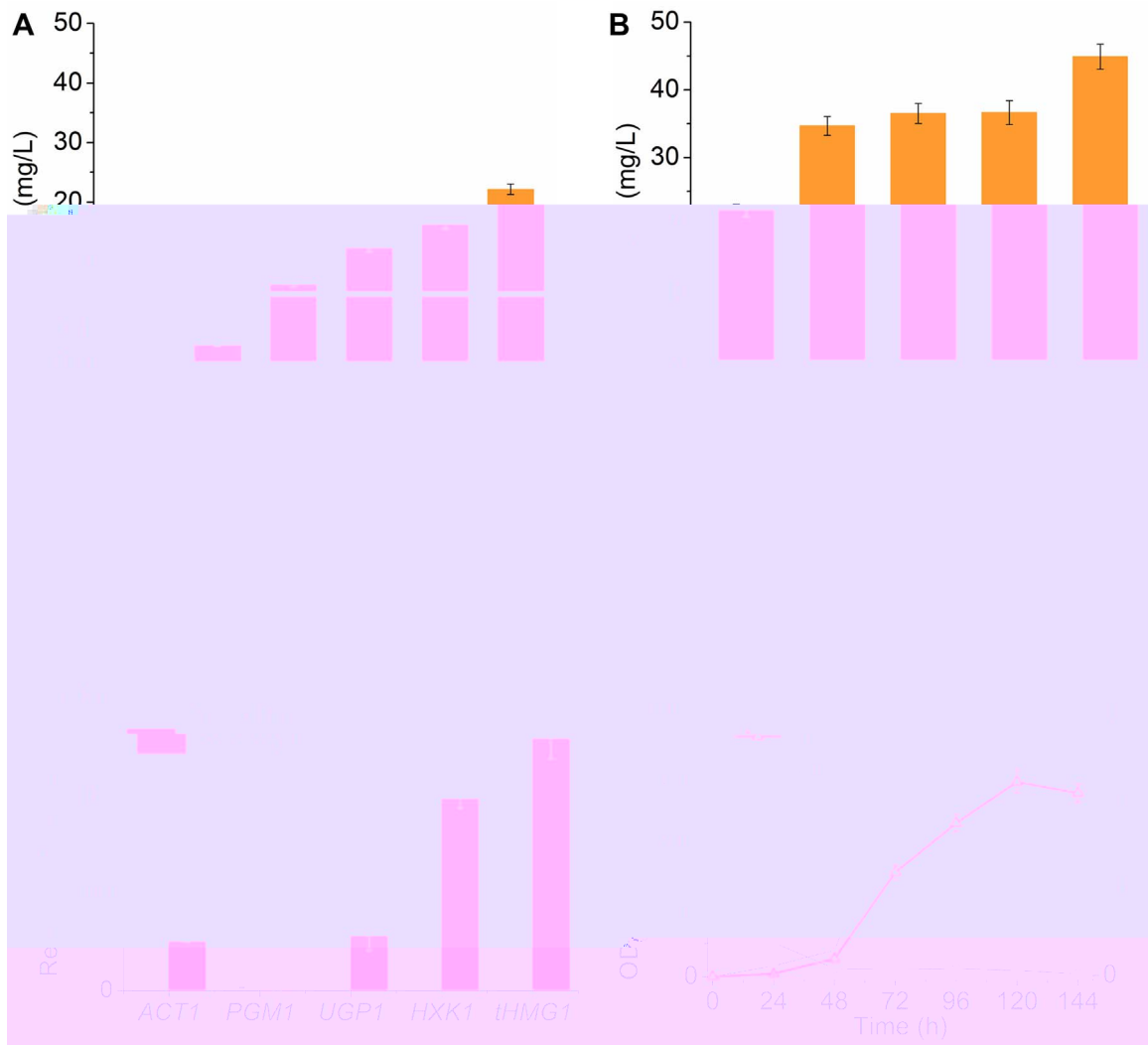


Fig. 5.

S. cerevisiae PGM1 A B
 UGP1 ACT1 PGM1 HXK1 HMG1
 D
 C
 ACT1
 PPDS-75 ATR2
 HXK1
 Δ
^tHMG1
 A D

Table 2

Strains	DCW	DDII ()	PPD ()	Rh2 ()
Δ				
Δ				
Δ				
Δ				
Δ				

PGM1 UGP1

S. cerevisiae

S. cerevisiae

Acknowledgements

EGH1
 ffi
 ffi in i o

Appendix A. Supporting information

References

E. coli

B. nap *S. cerei*

in vitro

S. cerei

ff

E. coli

fl

ff

F. proliferans

ff