Optimized microbial cells for production of melatonin and other compounds

Described herein are recombinant microbial host cells comprising biosynthetic pathways and their use in producing oxidation products and downstream products, e.g., melatonin and related compounds, as well as enzyme variants, nucleic acids, vectors and methods useful for preparing and using such cells. In specific aspects, the present invention relates to monoxygenases, e.g., amino acid hydroxylases, with a modified cofactor-dependency, and to enzyme variants and microbial cells providing for an improved supply of cofactors.

Glucose-based microbial production of the hormone melatonin in yeast *Saccharomyces cerevisiae*

Melatonin is a natural mammalian hormone that plays an important role in regulating the circadian cycle in humans. It is a clinically effective drug exhibiting positive effects as a sleep aid and a powerful antioxidant used as a dietary supplement. Commercial melatonin production is predominantly performed by complex chemical synthesis. In this study, we demonstrate microbial production of melatonin and related compounds, such as serotonin and N-acetylserotonin. We generated *Saccharomyces cerevisiae* strains that comprise heterologous genes encoding one or more variants of an L-tryptophan hydroxylase, a 5-hydroxy-L-tryptophan decarboxylase, a serotonin acetyltransferase, an acetylserotonin O-methyltransferase, and means for providing the cofactor tetrahydrobiopterin via heterologous biosynthesis and recycling pathways. We thereby achieved de novo melatonin biosynthesis from glucose. We furthermore accomplished increased product titers by altering expression levels of selected pathway enzymes and boosting co-factor supply. The final yeast strain produced melatonin at a titer of $14.50 \pm 0.57 \text{ mg L}^{-1}$ in a 76h fermentation using simulated fed-batch medium with glucose as sole carbon source. Our study lays the basis for further developing a yeast cell factory for biological production.
Melatonin, Serotonin, Microbial production, EasyClone vectors, Saccharomyces cerevisiae

Electronic versions:
Glucose_based_microbial_production_of_the_hormone_melatonin_in_yeast_Saccharomyces_cerevisiae.pdf

DOIs:
10.1002/biot.201500143
Transient overexpression of DNA adenine methylase enables efficient and mobile genome engineering with reduced off-target effects

Homologous recombination of single-stranded oligonucleotides is a highly efficient process for introducing precise mutations into the genome of E. coli and other organisms when mismatch repair (MMR) is disabled. This can result in the rapid accumulation of off-target mutations that can mask desired phenotypes, especially when selections need to be employed following the generation of combinatorial libraries. While the use of inducible mutator phenotypes or other MMR evasion tactics have proven useful, reported methods either require non-mobile genetic modifications or costly oligonucleotides that also result in reduced efficiencies of replacement. Therefore a new system was developed, Transient Mutator Multiplex Automated Genome Engineering (TM-MAGE), that solves problems encountered in other methods for oligonucleotide-mediated recombination. TM-MAGE enables nearly equivalent efficiencies of allelic replacement to the use of strains with fully disabled MMR and with an approximately 12- to 33-fold lower off-target mutation rate. Furthermore, growth temperatures are not restricted and a version of the plasmid can be readily removed by sucrose counterselection. TM-MAGE was used to combinatorially reconstruct mutations found in evolved salt-tolerant strains, enabling the identification of causative mutations and isolation of strains with up to 75% increases in growth rate and greatly reduced lag times in 0.6 M NaCl.
Microorganisms for the production of 5-hydroxytryptophan

Recombinant microbial cells and methods for producing 5-hydroxytryptophan (5HTP) using such cells are described. More specifically, the recombinant microbial cell comprises an exogenous gene encoding an L-tryptophan hydroxylase, and means for providing tetrahydrobiopterin (THB). Related sequences and vectors for use in preparing such recombinant microbial cells are also described.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, CFB - Core Flow, Bacterial Cell Factories
Authors: Knight, E. M. (Intern), Zhu, J. (Intern), Förster, J. (Intern), Luo, H. (Intern)
Publication date: 2013

Publication information
IPC: C12N1/21; C12N15/53; C12N15/55
Patent number: WO2013127914
Date: 06/09/2013

Bibliographical note
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Source: FindIt
Source-ID: 2287588966
Publication: Research - peer-review › Journal article – Annual report year: 2015

Scopus rating (2011): SJR 5.758 SNIP 2.172 CiteScore 7.86
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 5.24 SNIP 2.034
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 5.571 SNIP 1.869
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 4.641 SNIP 1.557
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 4.86 SNIP 1.787
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 4.55 SNIP 2.04
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 4.992 SNIP 2.152
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 4.809 SNIP 1.971
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 0.108 SNIP 1.862
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 0.12 SNIP 1.535
Scopus rating (2001): SJR 0.131 SNIP 1.402
Scopus rating (2000): SJR 0.141 SNIP 1.672
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 0.149 SNIP 1.562
Original language: English
Electronic versions:
Transient_overexpression_of_DNA_adenine_methylase_enables_efficient_and_mobile_genome_engineering_with_reduce_d_off_target_effects.pdf
DOIs:
10.1093/nar/gkv1090
Microorganisms for the production of melatonin

Recombinant microbial cells and methods for producing melatonin and related compounds using such cells are described. More specifically, the recombinant microbial cell may comprise exogenous genes encoding one or more of an L-tryptophan hydroxylase, a 5-hydroxy-L-tryptophan decarboxylase, a serotonin acetyltransferase, an acetylseryotonin O-methyltransferase; an L-tryptophan decarboxylase, and a tryptamine-5-hydroxylase, and means for providing tetrahydrobiopterin (THB). Related sequences and vectors for use in preparing such recombinant microbial cells are also described.

Projects:

Production of Human Metabolites in E.coli

Novo Nordisk Foundation Center for Biosustainability

CFB - Core Flow

iLoop

Bacterial Cell Factories

Period: 01/01/2013 → 30/09/2014
Number of participants: 3
Project participant:
Förster, Jochen (Intern)
Luo, Hao (Intern)
Zhu, Jiangfeng (Intern)