



US009410214B2

(12) **United States Patent**
Hawkins et al.

(10) **Patent No.:** **US 9,410,214 B2**
(45) **Date of Patent:** **Aug. 9, 2016**

(54) **USE OF PHOSPHOKETOLASE AND PHOSPHOTRANSACETYLASE FOR PRODUCTION OF ACETYL-COENZYME A DERIVED COMPOUNDS**

8,603,800 B2 12/2013 Gardner et al.
2012/0276587 A1 11/2012 Beck et al.
2013/0236942 A1 9/2013 Gardner et al.
2013/0330796 A1 12/2013 Beck et al.
2014/0154765 A1 6/2014 Gardner et al.

(71) Applicant: **Amyris, Inc.**, Emeryville, CA (US)

FOREIGN PATENT DOCUMENTS

(72) Inventors: **Kristy Michelle Hawkins**, Emeryville, CA (US); **Tina Tipawan Mahatdejkul-Meadows**, Emeryville, CA (US); **Adam Leon Meadows**, Emeryville, CA (US); **Lauren Barbara Pickens**, Emeryville, CA (US); **Anna Tai**, Emeryville, CA (US); **Annie Ening Tsong**, Emeryville, CA (US)

EP 2 546 336 A1 1/2013

OTHER PUBLICATIONS

(73) Assignee: **Amyris, Inc.**, Emeryville, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 172 days.

International Search Report and the Written Opinion in PCT/US2014/028421, mailed Sep. 19, 2014, 17 pages.
Byrne et al., The Yeast Gene Order Browser: Combining curated homology and syntenic context reveals gene fate in polyploid species (2005) *Genome Research* 15:1456-1461.
DeLuna et al., Need-Based Up-Regulation of Protein Levels in Response to Deletion of Their Duplicate Genes (2010) *PLoS Biology* 8:e10000347, 11 pages.
Hirayama et al., Cloning and characterization of seven cDNAs for hyperosmolarity-responsive (*HOR*) genes of *Saccharomyces cerevisiae* (1995) *Mol Gen Genet* 249:127-138.
Nevoigt et al., Osmoregulation and glycerol metabolism in the yeast *Saccharomyces cerevisiae* (1997) *FEMS Microbiol Rev* 21:231-241.
Norbeck et al., Purification and Characterization of Two Isoenzymes of *DL*-Glycerol-3-phosphatase from *Saccharomyces cerevisiae* (1996) *J Biol Chem* 271: 13875-13881.
Påahlman et al., The Yeast Glycerol 3-Phosphatases Gpp1p and Gpp2p Are Required for Glycerol Biosynthesis and Differentially Involved in the Cellular Responses to Osmotic, Anaerobic, and Oxidative Stress (2001) *J Biol Chem* 276: 3555-3563.
Sonderegger et al., Metabolic Engineering of a Phosphoketolase Pathway for Pentose Catabolism in *Saccharomyces cerevisiae* (2004) *Applied and Environmental Microbiology* 70:2892-2897.

(21) Appl. No.: **14/214,062**

(22) Filed: **Mar. 14, 2014**

(65) **Prior Publication Data**

US 2014/0273144 A1 Sep. 18, 2014

Related U.S. Application Data

(60) Provisional application No. 61/800,356, filed on Mar. 15, 2013.

(51) **Int. Cl.**

C12N 9/10 (2006.01)
C12N 9/16 (2006.01)
C12N 9/88 (2006.01)
C12P 5/00 (2006.01)
C12P 7/54 (2006.01)

(52) **U.S. Cl.**

CPC **C12Y 401/02009** (2013.01); **C12N 9/1029** (2013.01); **C12N 9/16** (2013.01); **C12N 9/88** (2013.01); **C12P 5/007** (2013.01); **C12P 7/54** (2013.01); **C12Y 203/01008** (2013.01); **C12Y 301/03021** (2013.01); **Y02P 20/52** (2015.11)

(58) **Field of Classification Search**

None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

7,253,001 B2 8/2007 Wahlbom et al.
8,221,982 B2 7/2012 Serber et al.
8,415,136 B1 4/2013 Gardner et al.

Primary Examiner — Suzanne M Noakes

(74) *Attorney, Agent, or Firm* — Squire Patton Boggs (US) LLP

(57) **ABSTRACT**

Provided herein are compositions and methods for improved production of acetyl-CoA and acetyl-CoA derived compounds in a host cell. In some embodiments, the host cell is genetically modified to comprise a heterologous nucleotide sequence encoding a phosphoketolase (PK), and a functional disruption of an endogenous enzyme that converts acetyl phosphate to acetate. In some embodiments, the host cell further comprises a heterologous nucleotide sequence encoding a phosphotransacetylase (PTA). In some embodiments, the enzyme that converts acetyl phosphate to acetate is a glycerol-1-phosphatase. In some embodiments, the glycerol-1-phosphatase is GPP1/RHR2. In some embodiments, the glycerol-1-phosphatase is GPP2/HOR2. The compositions and methods described herein provide an efficient route for the heterologous production of acetyl-CoA-derived compounds, including but not limited to, isoprenoids, polyketides, and fatty acids.

32 Claims, 11 Drawing Sheets