activities. Further, it produces NAD(P)H which is needed in the following fatty acid synthesis. The pyruvate dehydrogenase bypass route optionally together with an enhanced diacylglycerol acyltransferase activity as provided by the invention removes many bottlenecks in microbial lipid production.

The invention is directed to genetically modified fungal cells that have been modified to enhance the expression of a nucleic acid encoding PDC, ALD, ACS and/or DAT, and to methods of constructing them.

In particular the invention is directed to a genetically modified oleaginous fungal cell comprising: a)t ~~aleast one~~ nucleic acid with enhanced expression encoding ~~an enzyme selected from the group consisting of~~ pyruvate decarboxylase (PDC), and b) at least one nucleic acid with enhanced expression encoding an enzyme selected from the group consisting of acetaldehyde dehydrogenase (ALD), ~~and~~ acetyl-CoA synthetase (ACS) and diacylglycerol acyltransferase (DAT).

The invention is further directed to a genetically modified fungal cell comprising:

a) a nucleic acid with modified expression encoding a pyruvate decarboxylase (PDC) enzyme, and

b) at least one nucleic acid with modified expression encoding an enzyme selected from the group consisting of acetaldehyde dehydrogenase (ALD), acetyl-CoA synthetase (ACS) and diacylglycerol acyltransferase (DAT).

The invention is also directed to a genetically modified fungal cell comprising:

a) at least one nucleic acid with modified expression encoding an enzyme selected from the group consisting of pyruvate decarboxylase (PDC), acetaldehyde dehydrogenase (ALD) and acetyl-CoA synthetase (ACS), and

b) a nucleic acid with modified expression encoding a diacylglycerol acyltransferase (DAT) enzyme.

The invention is also directed to a method of producing lipids, comprising cultivating a~~the~~ genetically modified oleaginous fungal cell according to the invention in a medium containing carbon and nitrogen sources, and recovering the lipids produced.

The invention is further directed to a method of producing biofuel, or lubricant, said method comprising cultivating a~~the~~ genetically modified oleaginous fungal cell according to the invention in a medium containing carbon and nitrogen sources, and recovering the lipids produced, and optionally esterifying said lipids to obtain biodiesel or lubricant, or hydrogenizing the lipids to obtain renewable diesel or lubricant.

The invention is still further directed to a methods of preparing ~~i.e. constructing~~ an oleaginous~~genetically modified~~ fungal cell of the invention, said methods comprising transforming a fungal cell with

~~at least one nucleic acid with enhanced expression encoding an enzyme selected from the group consisting of pyruvate decarboxylase (PDC), acetaldehyde dehydrogenase (ALD) and acetyl-CoA synthetase (ACS); or with~~

a) a nucleic acid with enhanced expression encoding a pyruvate decarboxylase (PDC) enzyme, and

b) at least one nucleic acid with enhanced expression encoding an enzyme selected from the group consisting of acetaldehyde dehydrogenase (ALD), acetyl-CoA synthetase (ACS) and diacylglycerol acyltransferase (DAT)~~; or with~~

~~a) at least one nucleic acid with modified expression encoding an enzyme selected from the group consisting of pyruvate decarboxylase (PDC), acetaldehyde dehydrogenase (ALD) and acetyl-CoA synthetase (ACS), and~~

~~b) a nucleic acid with modified expression encoding a diacylglycerol acyltransferase (DAT) enzyme~~.

In addition the invention is directed to an expression cassette comprising

a) at least one nucleic acid with modified expression encoding an enzyme selected from the group consisting of pyruvate decarboxylase (PDC), acetaldehyde dehydrogenase (ALD) and acetyl-CoA synthetase (ACS), and

b) a nucleic acid with modified expression encoding a diacylglycerol acyltransferase (DAT) enzyme.

Conveniently the genetically modified fungal cells are constructed according to the invention by transforming the fungal cell with the nucleic acid(s) encoding said enzyme(s) resulting in enhanced enzyme activity of said enzyme.

Still further the invention is directed to an enzyme protein having phosholipid:diacylglycerol acyltransferase (PDAT) activity and at least 40% sequence identity to SEQ ID NO:52, or an enzymatically active fragment or variant thereof, and to an isolated nucleic acid molecule selected from the group consisting of: a) a nucleic acid encoding said protein, b) a nucleic acid comprising the nucleotide sequence of SEQ ID NO:53 or SEQ ID NO:93, c) a complementary strand of a) or b), and d) a sequence that is degenerate as a result of the genetic code to anyone of sequences a) - c).

The invention is additionally directed to a genetically modified fungal cell comprising a nucleic acid with modified expression encoding said PDAT, and to a

method of producing lipids, comprising cultivating said genetically modified fungal cell in a medium containing carbon and nitrogen sources, and recovering the lipids produced.

Still further the invention is directed to ~~the~~ use of a genetically modified fungal cell of the invention for producing lipids, precursors of functional fatty acids, functional fatty acids, biofuels, biodiesel, renewable diesel or lubricants. ~~The use for producing lipids includes e.g. the use for producing precursors of fatty acids e.g. of functional fatty acids, and for producing the fatty acids or functional fatty acids.~~

As a still further aspect, the invention is directed to a genetically modified *Cryptococcus* cell, which has been modified to enhance the expression of a heterologous nucleic acid, and to a method of constructing the cell, said method comprising transforming a *Cryptococcus* cell with a nucleic acid encoding a heterologous protein.

Specific embodiments of the invention are set forth in the dependent claims. Other objects, details and advantages of the present invention will become apparent from the following drawings, detailed description and examples.

Brief Description of the Drawings

Figure 1 shows probable metabolic routes for cytosolic acetyl-CoA production via the pyruvate dehydrogenase route in grey, and via the pyruvate dehydrogenase bypass route i.e. the pyruvate decarboxylase route in black.

Figure 2 shows the metabolic route for triacylglycerol production.

Figure 3 is a diagram depicting plasmid pKK81.

Figure 4 is a diagram depicting plasmid pKK82.

Figure 5 is a diagram depicting plasmid pKK86.

Figure 6 is a diagram depicting plasmid pKK95.

Figure 7 is a diagram depicting plasmid pKK85.

Figure 8 is a diagram depicting plasmid pKK75.

Figure 9 is a diagram depicting plasmid pKK94.

Figure 10 is a diagram depicting plasmid pKK96.

Figure 11 is a diagram depicting plasmid pKK98.

Figure 12 is a diagram depicting plasmid pKK101.

Figure 13 is a diagram depicting plasmid pKK102.