12-10-1996

Method for Obtaining Antifungal and Herbicidal Compounds that Target the First Committed Step in Shingolipid Long-Chain Base Biosynthesis

Robert C. Dickson
University of Kentucky, bobd@uky.edu

Robert L. Lester
University of Kentucky, rlester@email.uky.edu

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Follow this and additional works at: https://uknowledge.uky.edu/biochem_patents

Part of the Medical Biochemistry Commons

Recommended Citation
Dickson, Robert C. and Lester, Robert L., "Method for Obtaining Antifungal and Herbicidal Compounds that Target the First Committed Step in Shingolipid Long-Chain Base Biosynthesis" (1996). Molecular and Cellular Biochemistry Faculty Patents. 15. https://uknowledge.uky.edu/biochem_patents/15

This Patent is brought to you for free and open access by the Molecular and Cellular Biochemistry at UKnowledge. It has been accepted for inclusion in Molecular and Cellular Biochemistry Faculty Patents by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.
METHOD FOR OBTAINING ANTIFUNGAL AND HERBICIDAL COMPOUNDS THAT TARGET THE FIRST COMMITTED STEP IN SHINGOLIPID LONG-CHAIN BASE BIOSYNTHESIS

Inventors: Robert C. Dickson; Robert L. Lester, both of Lexington, Ky.

Assignee: The University of Kentucky Research Foundation, Lexington, Ky.

Appl. No.: 365,981

Filed: Dec. 28, 1994

Related U.S. Application Data


Int. Cl. C12N 1/15; C12N 1/19; C12N 1/21; C12N 5/10; C12N 15/54; C12N 15/63

U.S. Cl. 435/240.1; 435/252.3; 435/254.11; 435/320.1; 435/172.3; 435/193; 536/23.2

Field of Search 536/23.2, 23.74; 435/240.1, 252.3, 254.11, 256, 320.1, 193

References Cited

Nagiec et al., (1994) Proc. Nat. Acad. Sci., USA 91:

Primary Examiner—Robert A. Wax
Assistant Examiner—Gabriele E. Bugaisky
Attorney, Agent, or Firm—Lowe, Price, LeBlanc & Becker

ABSTRACT

The invention provides the LCB1 and LCB2 genes of the yeast Saccharomyces cerevisiae that encode subunits of the enzyme serine palmitoyltransferase (SPT), the first enzyme leading to synthesis of the long-base component of the sphingolipids. The present specification describes the isolation of the LCB1 and LCB2 genes. The invention further relates to methods of using these genes to either inhibit SPT activity or to inhibit synthesis of the enzyme. Furthermore, the invention relates to methods for constructing strains of S. cerevisiae or other organisms that can be used to select and to test for compounds that either inhibit SPT activity or to inhibit synthesis of the enzyme.

12 Claims, 11 Drawing Sheets
SERINE PALMITOYLTRANSFERASE:

\[
\text{CH}_3(\text{CH}_2)_4\text{CO-SCoA} + \text{HCN-CH}_2\text{OH} \xrightarrow{\text{PYRIDOXAL-P}} \text{CO}_2 + \text{CoA} + \text{CH}_3(\text{CH}_2)_4\text{CO-CH-CH}_2\text{OH}
\]

5-AMINOLEVULINIC ACID SYNTHASE:

\[
\text{HOOC(}\text{CH}_2)_2\text{CO-SCoA} + \text{HCN} \xrightarrow{\text{PYRIDOXAL-P}} \text{CO}_2 + \text{CoA} + \text{HOOC(}\text{CH}_2)_2\text{CO-CH}_2
\]

2-AMINO-3-KETOBUTYRATE LIGASE:

\[
\text{CH}_3\text{CO-SCoA} + \text{HCN} \xrightarrow{\text{PYRIDOXAL-P}} \text{CoA} + \text{CH}_3\text{CO-CH}_2
\]
FIGURE 5

A

NAME

<table>
<thead>
<tr>
<th>COMPLEMENT lcb2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COMPLEMENT</strong></td>
</tr>
<tr>
<td>- B7 +</td>
</tr>
<tr>
<td>- B7ΔS -</td>
</tr>
<tr>
<td>- 2.3 -</td>
</tr>
<tr>
<td>+ LCB2-R +</td>
</tr>
</tbody>
</table>

B

PHENOTYPE

lcb2A3::URA3 E URA3 E Lcb-

C

pRSLCB2-2

1 kb
**FIGURE 7B**

<table>
<thead>
<tr>
<th></th>
<th>SIVDHALINYNLITRN---TIVLKQETLPIVPSLKKICNAAMSPEELK</th>
<th>539</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCB1</td>
<td>SRVRFCMSA---SLTKED---IDYLLRVHSEVGDKLNKLKSGKSSYDGK</td>
<td>536</td>
</tr>
<tr>
<td>LCB2</td>
<td>NAVDDVFNELQLPRVREDWESQGGLGVESGFVEESNLWTSQSLTBD</td>
<td>527</td>
</tr>
<tr>
<td>YEAST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEM1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCB1</td>
<td>NA---CESVKQSLACQESN---K</td>
<td>558</td>
</tr>
<tr>
<td>LCB2</td>
<td>RQRWDEIEVIRRTPEDCKDDKFVN</td>
<td>561</td>
</tr>
<tr>
<td>HEM1</td>
<td>LNP---NVRDPVQLEVSQGIKQ</td>
<td>548</td>
</tr>
<tr>
<td>**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
METHOD FOR OBTAINING ANTIFUNGAL AND HERBICIDAL COMPOUNDS THAT TARGET THE FIRST COMMITTED STEP IN SHINGOLIPID LONG-CHAIN BASE BIOSYNTHESIS

This application is a continuation of application Ser. No. 07/906,899 filed Jun. 30, 1992, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to the isolation of the LCBl and LCB2 genes of the yeast Saccharomyces cervisiae that encode subunits of the enzyme serine palmitoyltransferase (SPT), the first enzyme leading to synthesis of the long-base component of sphingolipids. The invention further relates to methods of using these genes to either inhibit SPT activity or to inhibit synthesis of the enzyme. Furthermore, the invention relates to methods for construction strains of S. cervisiae or other organisms that can be used to select and test for compounds that either inhibit SPT activity or to inhibit synthesis of the enzyme.

2. Description of the Background


sphingolipids are derivatives of ceramides containing sugars and sometimes phosphates. Ceramides usually contain a fatty acid of 20-26 carbons connected via an amide linkage to a long-chain base. The major long-chain bases and their predominant distribution are:

Sphingosine (animals)
CH_{2}(CH_{2})_{14}-CH=CH-CH-CH_{2}OH
OH NH_{2}

Phytosphingosine (plants, fungi)
CH_{2}(CH_{2})_{14}-CH=CH-CH-CH_{2}OH
OH OH NH_{2}

The route of sphingolipid biosynthesis is proposed to be:

\[
\begin{align*}
\text{CH}_{2}(\text{CH}_{2})_{14}-\text{CH} & \equiv \text{CH} - \text{CH} - \text{CH}_{2} \text{OH} \\
\text{OH} & \text{NH}_{2} \\
\text{Phytosphingosine (plants, fungi)}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_{2}(\text{CH}_{2})_{14}-\text{CH} & \equiv \text{CH} - \text{CH} - \text{CH}_{2} \text{OH} \\
\text{OH} & \text{OH} \text{NH}_{2} \\
\text{Sphingosine (animals)}
\end{align*}
\]

The route of sphingolipid biosynthesis is proposed to be:

\[
\begin{align*}
\text{CH}_{2}(\text{CH}_{2})_{14}-\text{CH} & \equiv \text{CH} - \text{CH} - \text{CH}_{2} \text{OH} \\
\text{OH} & \text{NH}_{2} \\
\text{Phytosphingosine (plants, fungi)}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_{2}(\text{CH}_{2})_{14}-\text{CH} & \equiv \text{CH} - \text{CH} - \text{CH}_{2} \text{OH} \\
\text{OH} & \text{OH} \text{NH}_{2} \\
\text{Sphingosine (animals)}
\end{align*}
\]

The route of sphingolipid biosynthesis is proposed to be:

\[
\begin{align*}
\text{CH}_{2}(\text{CH}_{2})_{14}-\text{CH} & \equiv \text{CH} - \text{CH} - \text{CH}_{2} \text{OH} \\
\text{OH} & \text{NH}_{2} \\
\text{Phytosphingosine (plants, fungi)}
\end{align*}
\]

The route of sphingolipid biosynthesis is proposed to be:

\[
\begin{align*}
\text{CH}_{2}(\text{CH}_{2})_{14}-\text{CH} & \equiv \text{CH} - \text{CH} - \text{CH}_{2} \text{OH} \\
\text{OH} & \text{OH} \text{NH}_{2} \\
\text{Sphingosine (animals)}
\end{align*}
\]

The route of sphingolipid biosynthesis is proposed to be:

\[
\begin{align*}
\text{CH}_{2}(\text{CH}_{2})_{14}-\text{CH} & \equiv \text{CH} - \text{CH} - \text{CH}_{2} \text{OH} \\
\text{OH} & \text{OH} \text{NH}_{2} \\
\text{Sphingosine (animals)}
\end{align*}
\]

The route of sphingolipid biosynthesis is proposed to be:

\[
\begin{align*}
\text{CH}_{2}(\text{CH}_{2})_{14}-\text{CH} & \equiv \text{CH} - \text{CH} - \text{CH}_{2} \text{OH} \\
\text{OH} & \text{OH} \text{NH}_{2} \\
\text{Sphingosine (animals)}
\end{align*}
\]

The route of sphingolipid biosynthesis is proposed to be:

\[
\begin{align*}
\text{CH}_{2}(\text{CH}_{2})_{14}-\text{CH} & \equiv \text{CH} - \text{CH} - \text{CH}_{2} \text{OH} \\
\text{OH} & \text{OH} \text{NH}_{2} \\
\text{Sphingosine (animals)}
\end{align*}
\]

The route of sphingolipid biosynthesis is proposed to be:

\[
\begin{align*}
\text{CH}_{2}(\text{CH}_{2})_{14}-\text{CH} & \equiv \text{CH} - \text{CH} - \text{CH}_{2} \text{OH} \\
\text{OH} & \text{OH} \text{NH}_{2} \\
\text{Sphingosine (animals)}
\end{align*}
\]
may be vital for all organisms that contain them, and therefore, any compound that would inhibit long-chain base biosynthesis might inhibit growth of an organism that contained sphingolipids.

Accordingly, there is a need to begin to identify or design such inhibitory antifungal compounds to target the long-chain base biosynthesis pathway, which would appear to be a good target for antifungal compounds. Therefore we isolated two S. cerevisiae genes, LCB1 (SEQ ID NOS.: 1–3) and LCB2 (SEQ ID NOS.: 4–6), that most likely encode subunits of SPT. These are the first genes involved in long-chain base biosynthesis to be isolated from any organism. The genes provide a unique opportunity to identify compounds that block SPT activity or synthesis in specific organisms.

SUMMARY OF THE INVENTION

One objective of the present invention is to provide the LCB1 (SEQ ID NOS.: 1–3), and the LCB2 (SEQ ID NOS.: 4–6) genes of S. cerevisiae and to demonstrate that they provide SPT enzyme activity to a strain that lacks such enzyme activity.

Another objective of the present invention is to provide the LCB1 (SEQ ID NOS.: 1–3), and the LCB2 (SEQ ID NOS.: 4–6) genes of S. cerevisiae for use in constructing a genetically engineered strain of S. cerevisiae that has increased SPT protein and therefore enzyme activity.

Another objective of the present invention is to provide the DNA sequence of the LCB1 (SEQ ID NOS.: 1–3) and LCB2 (SEQ ID NOS.: 4–6) genes for use as targets for antisense or triple-helix-forming oligonucleotides which will inhibit the production of SPT protein.

Another objective of the present invention is to provide the DNA sequence of the LCB1 (SEQ ID NOS.: 1–3) and LCB2 (SEQ ID NOS.: 4–6) genes for use in overexpression of the genes and subsequent overproduction of the SPT enzyme.

Another objective of the present invention is to provide the DNA sequence of the LCB1 (SEQ ID NOS.: 1–3) and LCB2 (SEQ ID NOS.: 4–6) genes for use in isolating the homolog of these genes from other organisms.

Other objectives and advantages of the invention will become apparent as the description thereof proceeds.

In satisfaction of the foregoing objects and advantages, the present invention provides the LCB1 (SEQ ID NOS.: 1–3) and LCB2 (SEQ ID NOS.: 4–6) genes and their DNA sequence. The genes are shown to restore SPT activity to a lcb1(SEQ ID NOS.: 1–3)-defective and lcb2(SEQ ID NOS.: 4–6)-defective strain, respectively.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 represents a schematic diagram of plasmids carrying the LCB1 (SEQ ID NOS.: 1–3) gene of S. cerevisiae.

FIG. 2 sets forth the DNA sequence of the LCB1 (SEQ ID NOS.: 4–6) gene and the predicted protein product.

FIG. 3 sets forth a comparison of the LCB1 (SEQ ID NOS.: 1–3) protein sequence with other proteins that catalyze a chemical reaction that is similar to the one catalyzed by SPT.

FIG. 4 sets forth a comparison of the reaction catalyzed by SPT and other enzymes.

FIG. 5(A–C) represents a schematic diagram of plasmids carrying the LCB2 (SEQ ID NOS.: 4–6) gene of S. cerevisiae or portions of the gene.

FIG. 6 sets forth the DNA sequence of the LCB2 (SEQ ID NOS.: 4–6) gene and the predicted protein sequence.

FIG. 7 sets forth a comparison of LCB1 (SEQ ID NOS.: 1–3), LCB2 (SEQ ID NOS.: 4–6), and the S. cerevisiae HEM1 protein sequences.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention relates to the isolation of the LCB1 (SEQ ID NOS.: 1–3) and LCB2 (SEQ ID NOS.: 4–6) genes of S. cerevisiae.

The present invention provides a DNA sequence LCB1 having a nucleotide sequence as set forth in FIG. 2. It also provides a plasmid comprising the LCB1 sequence according to the invention. Particularly preferred is a plasmid according to the invention which is the plasmid pTZ18-LCB1 (SEQ ID NOS.: 1–3) containing the LCB1 (SEQ ID NOS.: 1–3) sequence. Also, particularly preferred is a plasmid according to the invention which is plasmid YplLCB1-1 containing the LCB1 sequence.

In another embodiment the present invention provides a host cell line transformed by a plasmid containing the LCB1 (SEQ ID NOS.: 1–3) sequence according to the present invention.

In another aspect the present invention provides a DNA sequence LCB2 (SEQ ID NOS.: 4–6) having a nucleotide sequence as set forth in FIG. 6. It also provides a plasmid comprising the LCB2 (SEQ ID NOS.: 4–6) sequence according to the invention. Particularly preferred is a plasmid according to the invention which is the plasmid pRSLCB2-2 containing the LCB2 (SEQ ID NOS.: 4–6) sequence.

In another embodiment the present invention provides a host cell line transformed by a plasmid containing the LCB2 (SEQ ID NOS.: 4–6) sequence according to the present invention.

The present invention further provides a genetically engineered strain of S. cerevisiae which has increased production of Serine Palmitoyltransferase protein and therefore increased enzyme activity as compared to the wild type S. cerevisiae.

In another aspect the present invention provides an antisense or triple helix forming oligonucleotide specific for the LCB1 (SEQ ID NOS.: 1–3) sequence, which will inhibit the production of Serine Palmitoyltransferase protein.

In still another aspect the present invention provides an antisense or triple-helix-forming oligonucleotide specific for the LCB2 (SEQ ID NOS.: 4–6) sequence, which will inhibit the production of Serine Palmitoyltransferase protein.

The present invention also provides a genetically engineered microbial strain transformed by a plasmid comprising either the LCB1 (SEQ ID NOS.: 1–3) or LCB2 (SEQ ID NOS.: 4–6) sequence, or both the LCB1 (SEQ ID NOS.: 1–3) and LCB2 (SEQ ID NOS.: 4–6) sequences, which overexpresses the gene(s) with which it is transformed and subsequently overproduces the Serine Palmitoyltransferase enzyme.

Also the present invention provides a method for testing an oligonucleotide or organic compound for the ability to block Serine Palmitoyltransferase activity or synthesis, which method comprises:

exposing the oligonucleotide or the organic compound being tested to a host cell or host cell extract, which host cell has been transformed to include either a LCB1 (SEQ ID
NOS.: 1–3) gene or LCB2 (SEQ ID NOS.: 4–6) gene (or both genes), and

testing for an absence of Serine Palmitoyltransferase enzyme or its activity, which diminished activity is indicated by the absence or lower concentration of sphingolipids.

The present invention further provides an oligonucleotide DNA sequence, which is a complement to either the LCB1 (SEQ ID NOS.: 1–3) or LCB2 (SEQ ID NOS.: 4–6) sequences, or to portions thereof.

In yet another aspect the present invention provides a method of testing for and/or isolating closely related sequences (similar to LCB1 (SEQ ID NOS.: 1–3)) which comprises

producing or obtaining an oligonucleotide which is a complement to a portion of the LCB1 (SEQ ID NOS.: 1–3) gene, and

using the complement as an oligonucleotide probe by

exposing a target nucleotide sequence to the said nucleotide probe and testing for binding to said probe, and optionally

isolating and separating the nucleotide probe from the DNA sequence to which it has bound.

In still another aspect, the present invention provides a method of testing for and/or isolating closely related sequences (similar to LCB2 (SEQ ID NOS.: 4–6)) which comprises

producing or obtaining an oligonucleotide which is a complement to a portion of the LCB2 (SEQ ID NOS.: 4–6) gene, and

using the complement as an oligonucleotide probe by

exposing a target nucleotide sequence to the said nucleotide probe and testing for binding to said probe, and optionally

isolating and separating the nucleotide probe from the DNA sequence to which it has bound.

The LCB1 (SEQ ID NOS.: 1–3) and LCB2 (SEQ ID NOS.: 4–6) sequences according to the present invention, plasmids comprising either of the LCB1 (SEQ ID NOS.: 1–3) or LCB2 (SEQ ID NOS.: 4–6) sequences, transformed host cells having a sequence according to the present invention, and sequences which are complements are all useful in screening potential antifungal agents, or for producing reagents useful in screening potential antifungal agents, (both oligonucleotides and organic chemical agents, which are potential antifungal agents may be screened).

The sequences according to the present invention are also useful to provide oligonucleotides which have complementary DNA sequences, which complementary sequences can be used as probes to screen for sequences which are homologous of the claimed sequences and used in a process to isolate and ultimately sequence such homologs of LCB1 (SEQ ID NOS.: 1–3) or LCB2 (SEQ ID NOS.: 4–6).

In accordance with present invention, as a preliminary step, a mutant strain of *S. cerevisiae* blocked in sphingolipid biosynthesis was obtained. For example, strains of *S. cerevisiae* carrying the mutant allele, leb1-1, are absolute auxotrophs and grow only when a long-chain base (leb, phytosphingosine but not sphingosine) is added to the culture medium.

The genes were isolated from a *S. cerevisiae* genomic DNA library by complementation for growth on medium lacking a long-chain base (such as phytosphingosine) of an lel6b-defective strain.

The original leb mutant MCGA (MATa lebl-1 inf1 (J. Biol. Chem. 258, 10200–10203 (1983) was crossed with strain W303-1B (MATα ade2-1 can1-100 ura3-1 his3-11,15 trp1-1 leu2-3,112; obtained from R. J. Rothstein, Columbia University). Progeny from this cross were backcrossed to W303-1B, and several offspring were selected for further study, including strains X2A1B (MATa lebl-1 ura3-1 trpl-1 his3-11,15). Strain SL1 was derived from strain SJ21R (MATa ura3-52 leu2-3,112 ade1 MEL1) by replacement of the LCB1 allele with a mutant allele that was disrupted by inserting a 1.1 kb URA3 DNA fragment at the Sall site of LCB1. The LCB1:: URA3-disrupted allele was prepared by transferring 4.3 kb HindIII-Stu fragment, carrying LCB1, from plCB2 to pTZ18 (Pharmacia) cleaved with HindIII and SmaI. The resulting plasmid, pTZ18-LCB1 was cleaved with Sall and ligated with a 1.1-kb URA3 DNA fragment having Sall cohesive ends (obtained from pUC-URA3 cut with Sall) to yield pTZ18-LCB1:: URA3.

To replace the LCB1 chromosomal allele with the URA3 disrupted allele, 10 μg of pTZ18-LCB1:: URA3 DNA was cleaved with XbaI and Clal, extracted with phenol, phenol-chloroform, and chloroform and precipitated with ethanol. The DNA was transformed into strain SJ21R with selection for Ura+ transformants. Replacement of the LCB1 chromosomal allele with the URA3 disrupted allele was verified by Southern blot analysis. YipLCB1-1 was constructed by inserting TRP1 of *S. cerevisiae*, as a 1.4-kb HindIII fragment into the HindIII site of pTZ18-LCB1. YipLCB1-1

was cleaved at its unique BamHI site located on the 3′ side of LCB1, and the linear DNA was used to transform strain 2G45 with selection for Ura+ transformants. Integration at the expected chromosomal location was verified by Southern hybridization. Transformants were crossed to strain YPH1 (MATa ura3-52 lys2-801 ade2-101 (See, for example, Genetics, 122, 19–27 (1989)).

The plasmid pLCB was isolated from a *S. cerevisiae* genomic DNA library carried in a CEN vector. The 6.44-kb vector was pBR322 with a 0.63-kb Sau3A CEN3 DNA fragment inserted into the PvuII site of the vector and a 1.4-kb TRP1 ARS1 fragment inserted into the EcoRI site of the vector. The ligations were done with molecules whose ends were made blunt ended so that the original restriction sites were destroyed. Sau3A genomic DNA fragments of 8-kb average size from strain X2180 (αααα gal2/gal2) were cloned into the BamHI site of the vector (the library was obtained from ZymoGenetics, Seattle, Wash.). DNA fragments from pLCB were subcloned into YCp50 (see, Methods Enzymol., 152, 481–504 (1987)).

Plasmids were propagated in *Escherichia coli* DH5α. The leb-defective strains were propagated in several media as described later in the detailed section which follows.

For example, to isolate LCB1, strain X2A1B (relevant genotype leb1-1, trpl) was transformed with a genomic DNA library which was carried in a vector containing CEN3 and ARS1, for single-copy propagation in yeast cells, and TRP1, for selection of Trp+ yeast that had been transformed with the vector. Ten thousand Trp+ transformants were selected on minimal medium plates containing phytosphingosine but lacking tryptophan. Transformants were pooled and reselected on minimal medium plates lacking both tryptophan and phytosphingosine. About one per thirty-five hundred Trp+ colonies was able to grow without added phytosphingosine and thus had an Lcb+ phenotype.

Plasmid DNA was isolated from several Lcb+ yeast transformants and transformed into *E. coli* with selection for ampicillin resistant cells. Plasmid DNA from *E. coli* transformants was isolated and digested with restriction endonucleases. The pattern of restriction fragments indicated that
the original Lcb+ yeast transformants all contained the same plasmid which carried an insert of about 8 kb.

To localize the LCB1 gene on the 8 kb DNA insert we subcloned parts of the insert into the CEN4 vector Ycp50 and tested the resulting plasmids for their ability to confer a Lcb+ phenotype on strain X2A1B. The experiments localized LCB1 to a subclone of 4.0 kb (FIG. 1).

Further localization of LCB1 was achieved by chromosomal disruption. For these experiments the 4 kb insert was disrupted at the unique Sall site (FIG. 1) by insertion of the URA3 gene of *S. cerevisiae* to create the lecb::URA3-disruption allele. The lecb::URA3-disruption allele was used to replace the wild type LCB1 allele in strain SJ21R (relevant phenotype Lcb+ Ura-) by homologous recombination as described in EXAMPLE 2. These procedures produced a strain, SL1, having the chromosome disrupted at the expected Sall site. If this procedure had disrupted the LCB1 gene then the strain SL1 should require long-chain base (phytosphingosine) for growth and, therefore, having a Lcb- phenotype. This expectation was verified because strain SL1 had a Lcb- phenotype. We conclude that the Sall site shown in plasmid Ycp50-LCB1 between the Psl and Hpal sites is located within the LCB1 gene.

Genetic complementation analysis was used to verify that the lecb::URA3 disruption mutation in strain SL1 was allelic to the original lecb-1 mutation carried in strain X2A1B. Strain SL1 was crossed to strain 24D5. The resulting diploids had an Lcb- phenotype, suggesting allelism of the cloned gene and lecb1. Strong support for allelism was obtained by sporulating these diploids and showing that all tetrads give four Lcb- spores. However, such diploids failed to sporulate under a variety of conditions suggesting that sphingolipids are needed for sporulation. An alternative genetic approach was used to demonstrate allelism. The putative LCB1 allele, carried on the integrating vector YplLCB1-1, was directed to integrate into its homologous chromosomal locus as described in EXAMPLE 3. The host strain for integration of YplLCB1-1 was strain 24D5 which carried the lecb-1 mutation. If YplLCB1-1 did indeed carry the wild type LCB1 gene then the host strain should have this plasmid integrated next to the lecb-1 allele. When this strain is crossed to an LCB1 strain (YPH11) all progeny should be Lcb- since YplLCB1-1 should be tightly linked to lecb-1 and there should be little if any recombination events that would separate the two alleles. In fourteen-four spored tetrads from such a cross, showing 2:2 segregation for the Ade. Ura and Leu phenotypes, all spores were Lcb- indicating that YplLCB1 had been directed to integrate in close proximity to the lecb-1 allele. We conclude that the LCB1 gene has been cloned and is carried on pTZ18-LCB1 gene as claimed.

To determine if SPT activity was missing in lecb-1-defective strains and to determine if a plasmid carrying LCB1 restored such activity we assayed membranes for the enzyme. The parental strain MC6A contained 54.4 units of enzyme activity per mg of protein while the lecb-1-defective strain X2A1B contained 2.5 units per mg of protein or about 20 times less enzyme activity that the parental strain: this level of activity is at the limit of detection and the actual enzyme activity may be lower. The cloned LCB1 allele carried in pLCB1 was able to restore enzyme activity to about 50% of the wild-type level since three independent transformants of strain X2A1B gave 22.7, 25.6, and 22.8 units of enzyme activity per mg of protein. These data support the claim that LCB1 encodes the SPT enzyme or a subunit of the enzyme.

Based upon the results of the lecb1::URA3 disruption experiments a region surrounding the Sall site shown in FIG.

I was subjected to DNA sequence analysis and the sequence was analyzed by computer to locate large open reading frames which could encode the LCB1 protein. The sequence (FIG. 2) contained a single, large open reading frame, encoding 558 amino acids which was oriented in the same direction of transcription as the LCB1 mRNA (data not shown). This region must code for the LCB1 protein product because it is in the correct 5' to 3' orientation, because a URA3 disruption of the open reading frame at the unique Sall site created a Lcb- phenotype, and because it is genetically tightly linked to the lecb1-1 allele.

The nucleotide sequence of the open reading frame was used to produce the amino acid sequence of the LCB1 peptide. The results of the prediction are illustrated above each codon of the nucleotide sequence (FIG. 2) beginning with the first ATG codon at position +1 and ending with the stop codon TAA at position +1675. Assuming that this ATG codon is the true translation initiation site, the product of the open reading frame is a protein of 558 amino acids. Since the amino terminus of the LCB1 protein has not been determined directly it is possible that the amino terminus of the actual protein is different than indicated in FIG. 2. The difference could occur either because of post-translational processing or because an ATG codon down stream of the one shown in FIG. 3 is used as the initiation codon.

Because SPT activity is present in the membrane fraction of lyzed cells, we expected the LCB1 protein to be membrane-associated. The hydrophobicity of the deduced protein sequence was therefore examined for potential membrane spanning regions. According to the Theory of Kyte, J., and Doolittle, R. F. 1982. *J. Mol. Biol.* 157:105–132, the Grand Average Hydropathy Score (GRAVY) for the predicted LCB1 protein is −1.39, a value that places the protein in the same class as globular proteins. A globular, rather than integral membrane, protein is also predicted by the procedure of Eisenberg, D., Schwartz, E., Komaromy, M., and Wall, R. 1984. *J. Mol. Biol.* 179:125–142. In addition, this analysis predicts two very hydrophilic, membrane-associated helices. Helix I spans amino acid residues 12–32 and has the sequence IPIPAFIVTTSYSLYWFIVNLW while Helix II spans residues 344–373 and has the sequence ATADITVGSMTLAGSTGCGFVGL.


The similarity of the LCB1 protein to ALA synthase and to 2-amino-3-ketobutyrate CoA ligase seems particularly significant since these enzymes catalyze a reaction (FIG. 4) that is very similar to that catalyzed by SPT. In addition, the *E. coli* 2-amino-3-ketobutyrate CoA ligase uses pyridoxal...
phosphate as a cofactor (Mukherjee, J. J., Dokker, E. E. 1987. *J. Biol. Chem.* 262:14441-14447) as do serine palmitoyltransferase (Brady, R. O. and Koval, G. J. 1957. *J. Am. Chem. Soc.* 79:2648-2649) and ALA synthase (Warnich, G. R., and Burnham, B. F. 1971. *J. Biol. Chem.* 246:6880-6885). The similarity of the amino acid sequences (FIG. 3) and the reactions catalyzed by these enzymes (FIG. 4) argue that the product of LCB1 is most likely SPT or a catalytic subunit of the enzyme, rather than a regulatory protein that regulates transcription of LCB1 or the enzymatic activity of SPT.

Besides lcb1-mutant strains, lcb2-mutant strains also lack SPT enzyme activity (Pinio, W. J., Wells, G. W., and Lester, R. L. 1992. *J. Bacteriol.* 174:2575-2581). The LCB2 gene was isolated from a *S. cerevisiae* genomic DNA library of complementation for growth on medium lacking phytosphingosine of the lcb2 mutation carried in strain BS238. The strain was transformed with the same recombinant DNA library that was used for isolation of LCB1. *Ura* transformants were selected, pooled, and replated on plates lacking phytosphingosine to select transformants that could grow in the absence of phytosphingosine (*Lcb*'). Plasmid DNA was recovered from *Lcb*' cells by transfection into E. coli. Plasmid DNA isolated from E. coli was analyzed by restriction digestion. The pattern of restriction fragments indicated that all plasmids carried the same insert of about 7-kb which we designated B7 (FIG. 5).

LCB2 was localized by subcloning and testing the subclones for their ability to complement the lcb2 mutation in strain BS238 and allow the strain to grow in the absence of phytosphingosine (EXAMPLE 4). These data localized the LCB2 gene to a region near the Apal site shown in FIG. 1. DNA around this site was sequenced and the sequence was scanned by computer in all reading frames. There was only one large open reading frame, indicated by the open box at the top of FIG. 5. The determined DNA sequence and the translated open reading frame representing the putative LCB2 protein are indicated in FIG. 6.

To prove that this open reading was the LCB2 gene we used the cloned gene to make a chromosomal deletion allele lcb2A23;URA3 (EXAMPLE 5), as shown in FIG. 5. The deletion allele was originally introduced into the diploid strain YPH501 and Southern blotting was used to verify that the deletion strain carried one normal allele and the deletion allele (data not shown). The diploid was sporulated and spores were tested for their Lcb phenotype. All 17 four-spored tetrads showed 2:2 segregation for the Lcb"/Lcb" phenotype and all the Lcb" spores were Ura" as expected for a URA3 gene disruption. Thus, the deleted region is needed for long-chain base synthesis as would be expected if the region was the LCB2 gene. To verify that the putative LCB2 gene indicated in FIG. 5 is allelic to the authentic LCB2 gene, we used the integrating vector pRSLCB2-2 (EXAMPLES 6 and FIG. 5) which only carries the 5's half of the putative LCB2 gene. The plasmid was directed, by digestion with NsiI, to integrate into the genome of strain BS238 (relevant genotype lcb2), at the homologous NsiI site located in the putative LCB2 gene. Integration at the correct chromosomal location was verified by Southern blotting (data not shown). The strain carrying the integrated plasmid was crossed to strain YPH-500, diploids were selected, and sporulated. Twenty-five four-spored tetrads gave 2 Lcb"/2 Lcb" segregation and all of the Lcb" spores were Leu" while the Lcb" spores were Leu". These data demonstrate that the cloned DNA fragment directs integration at or near the lcb2 allele carried in strain BS238. Taken as a whole the data demonstrate that the LCB2 gene has been cloned.

The predicted sequence of the LCB2 protein is shown in FIG. 6. The protein contains 561 amino acid residues. Since the amino terminus of the LCB2 protein has not been determined directly it is possible that the amino terminus of the actual protein is different than indicated in FIG. 6. The difference could occur either because of post-translational processing or because an ATG codon down stream of the one shown in FIG. 6 is used as the initiation codon. A membrane-associated helix is predicted for residues 57 to 77 (PYYISLTVLYNL) and 443-463 (LGFIVYGVAD-SPVPIPLLWCP) by the algorithm of Eisenberg et al., (1984).

Comparison of the LCB2 protein sequence against other sequences in GenBank using the FASTA search procedure of Pearson, W. R. and Lipman, D. J., 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444-2448 revealed that the sequence was homologous to the LCB1 protein and to various ALA synthases including the one from *S. cerevisiae* (FIG. 7). In addition, the sequence was homologous to the BAC-8007X (Genbank) and the ECOB1LTDH (Genbank, called EKBL (SEQ ID NO.: 8) in FIG. 3) sequences (data not shown).

The similarity of the LCB2 protein to the ALA synthases and to 2-amino-3-ketobutyrate CoA ligase (EKBL, FIG. 3, ECOB1LTDH Gen Bank) seems particularly significant since these enzymes catalyze a reaction (FIG. 4) that is very similar to that catalyzed by SPT. In addition, the *E. coli* 2-amino-3-ketobutyrate CoA ligase uses pyridoxal phosphate as a cofactor (Mukherjee and Dekker, 1987) as do serine palmitoyltransferase and ALA synthase. The similarity of the amino acid sequences (FIG. 6) and the reactions catalyzed by these enzymes (FIG. 4) argue that the product of LCB2 is most likely SPT or a catalytic subunit of the enzyme, rather than a regulatory protein that regulates transcription of LCB2 or the enzymatic activity of SPT.

Potential uses of the LCB1 and LCB2 genes.

One use of the genes is to construct strains of *S. cerevisiae* or other organisms or cell lines that can be used to screen for inhibitors of SPT enzyme activity or inhibitors of expression of the LCB1 or LCB2 gene at the transcriptional or translational level. To construct a strain for screening inhibitors of SPT activity, one can use the LCB1 and LCB2 genes to overproduce their protein product. Overproduction will yield a host organism relatively more resistant to SPT inhibitors compared to a host that does not overproduce the proteins. This principle was first demonstrated in *S. cerevisiae* by Rine, J., Hansen, W., Hardeman, E., and Davis, R. W. 1983. *Proc. Natl. Acad. Sci. U.S.A.* 80:6750-6754. In the case of an inhibitor of transcription or translation, for example a triple helix-forming oligonucleotide or an antisense oligonucleotide, one can construct a strain carrying multiple copies of the LCB1 and LCB2 genes. Multiple copies should make the strain more resistant to the inhibitor than a strain having only one copy of each gene. A variation of this approach could be used for inhibitors of translation (an antisense oligonucleotide) in which the LCB1 and LCB2 coding regions would be fused to a strong promoter-enhancer region so that a single copy of the fusion genes would give high levels of LCB1 and LCB2 mRNA.

Another use of the LCB1 and LCB2 genes is to overexpress them and overproduce their protein product. Such overproduction usually makes it possible to purify the proteins. Expression and overproduction could be achieved in any number of organisms including *E. coli, S. cerevisiae,* or insect cells or other hosts for baculovirus vectors. The purified protein could then be used to identify or design inhibitors of SPT enzyme activity.
Finally, the LCB1 and LCB2 genes can be used to isolate their homologs from other organisms. Homologs can be isolated by complementation of the lcb1 and lcb2 mutation in appropriate *S. cerevisiae* host strains as those presented in this application. Alternatively, degenerate primers for the polymerase chain reaction (PCR) could be designed based upon the sequence of LCB1 and LCB2 and used to prime a PCR reaction using genomic or cDNA from the organism whose LCB genes are to be cloned. LCB1 and LCB2 homologs from particular organisms would enable the design of highly specific triple-helix forming or antisense oligonucleotides or for inhibitors of SPT activity unique to a particular organism.

In the examples the following materials were used:

*S. cerevisiae*: The original lcb mutant MC6A (MATa lcb1-1 inol; Wells and Lester, 1983), was crossed with strain W303-1B (MATa ade2-1 can1-100 ura3-1 his3-11,15 trp1-1 leu2-3,112; obtained from R. J. Rothstein, Columbia, Univ.). Progeny from this cross were backcrossed to W303-1B, and several offspring were selected for further study including strains Y2A1B (MATa lcb1-1 ura3-1 trp1-1 his3-11,15) and 24D5 (MATa lcb1-1 ura3-1 trp1-1 leu2-3,112 his3-11,15). Strains YPH1 (MATa ura3-52 lys2-801 ade2-101), YPH500 (MATa ura3-52 leu2-801amber leu2-D1011 alpha1 trp1-D63 his3-20 leu2-D1), and YPH501 (MATa ura3-52 leu2-801amber leu2-1011alpha1 trp1-D63 his3-20 leu2-D1) were obtained from Sikorski, R. S. and Hieter, P., 1989, *Genetics*, 122:19–27. Strain BS328 (MATa lcb2 ura3-52 leu2-3,112 ade1) was from Pinto, W. J., Srivivasan, B., Shepherd, S., Schmidt, A., Dickson, R. C., and Lester, R. L., 1992, *J. Bacteriol*, 174:2565–2574. Strain SIJ21R (MATa ura3-52 leu2-3,112 ade1 MELI) was described in Dickson, R. C., Wells, G. B., Schmidt, A., and Lester, R. L., 1990, *Mol. Cell. Biol.*, 10:2176–2181. The YPH strains are sensitive to the long-chain base phosphoshingosine and in order to transform them with DNA it is necessary to use 12.5 μM phosphoshingosine and 0.025% tritogal (half of the normal concentrations) in selection plates. Likewise, for genetic crosses involving YPH strains it is necessary to make the same adjustments for dissolution plates (minimal medium, Sherman, Fink, G. R., and Hicks, T. B. 1986, *Methods in Yeast Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) otherwise spores will not germinate.

*Escherichia coli*: strain DH5α was used for propagation of plasmids.

Media: PYED contained 1% peptone, 1% yeast extract, 2% agar (for plates), 50 mM sodium succinate (pH 5), isonitrol (50 mg/g), potassium phosphate monobasic (50 mg/ml), and 2% or 4% glucose. Minimal medium contained 1x Difco Yeast Nitrogen Base without amino acids, 50 μM sodium succinate (pH 5), 2% glucose, 1.5% agar (for plates), 50 μg/ml, valine (150 mg/ml), isoleucine (30 mg/ml), threonine (200 mg/ml) and these supplements at 20 mg/ml: adenine sulfate, arginine-HCl, histidine-HCl, leucine, lysine-HCl, methionine, tryptophan, and uracil. One or more supplements were omitted from minimal medium for selection of yeast transformants. For strains requiring long chain base the medium was supplemented with 25 μM phosphoshingosine (Sigma, St. Louis, Mo.). A 10x stock solution of phosphoshingosine was prepared by adding 0.25 ml of 100 μM phosphoshingosine (dissolved in 95% ethanol) to 99.75 ml of a 0.5% solution of tertogal (Sigma, St. Louis, Mo.).

DNA sequencing: Synthetic oligonucleotide primers were used forideoxynucleotide sequencing with Sequenase Version 2.0 DNA Polymerase (USB, Cleveland, Ohio) essentially as recommended by the supplier. The LCB1 sequence (FIG. 2) has been deposited in the Gen Bank and given accession number M63674. The LCB2 sequence (FIG. 6) has been deposited in the Gen Bank and given accession number M95669.


**EXAMPLE 1**

The plasmid pLCB (FIG. 1) was isolated from a *S. cerevisiae* genomic DNA library carried in a vector containing the CEN3 region of *S. cerevisiae* DNA. The 6.44 kb vector was pBR322 with a 0.63 kb Sau3A CEN3 DNA fragment inserted into the Prv1 site of the vector and a 1.4 kb TRP1ARS1 fragment inserted into the EcoRI site of the vector. These ligation were done with molecules whose ends were made blunt-ended so that the original restriction sites were destroyed. Sau3A genomic DNA fragments of 6.44 kb average size from strain X2180 (a/a gal2 gal1) were cloned into the BamHI site of the vector (the library was a gift from Zymogenetics, Seattle, Wash.). To construct YCP50-LCB1, a 4.7 kb Stul fragment from pLCB containing the LCB1 region, was subcloned into the NruI site of YCP50 (Rose, M. D. 1987. *Meth. Enzymology*. 152:481–504).

**EXAMPLE 2**

Strain SL1 as derived from strain SIJ21R by replacement of the LCB1 allele with a mutant allele that was disrupted by inserting a 1.1 kb URA3 DNA fragment from *S. cerevisiae* into the Sall site of LCB1 (FIG. 1 shows the Sall site). The lcb1::URA3 - disrupted allele was prepared by ligating a 4.3 kb HindIII-Stul fragment, carrying LCBl, derived from pLCB (FIG. 1) to pLTZ18 (Pharmacia) which had been cleaved with the restriction endonucleases HindIII and Smal. The resulting plasmid, pLTZ18-LCB1 (FIG. 1), was cleaved with Sall and ligated with a 1.1 kb URA3 DNA fragment having Sall cohesive ends to yield pLTZ18-LCB1::URA3. To replace the LCB1 chromosomal allele with the URA3-disrupted allele, ten micrograms of pLTZ18-LCB1::URA3 DNA was cleaved with XbaI and Clal, extracted with phenol, phenol:chloroform and chloroform, and precipitated with ethanol. The DNA was transformed into strain SIJ21R with selection for Ura" transformants. Replacement of the LCB1 chromosomal allele with the URA3-disrupted allele was verified by Southern blot analysis. Total DNA isolated from SL1 and the non-disrupted parental strain SIJ21R was cleaved with the restriction endonucleases NruI and Stul. Following Southern blot analysis, the parental strain showed a 4 kb band of hybridization, as expected, when the blot was probed with a 32P-labeled NruI to Stul DNA probe containing the LCB1 region (FIG. 1). If the lcb1::URA3-disrupted allele had replaced the wild type allele of LCB1 in strain SL1 then the Southern blot of strain
SL1 should show two bands that hybridize to the $^{32}$P-probe because URA3 contains a SstI cleavage site. The fragments should be 2.1 kb and 3 kb in length. The Southern blot (data not shown) contained the two expected bands of hybridization indicating that strain SL1 carried the leb1::URA3- disruption allele.

EXAMPLE 3

YIpLCB1-1 was constructed by inserting TRP1 of S. cerevisiae, as a 1.4 kb Hind III fragment, into the Hind III site of pTZ18-LCB1. YIpLCB1-1 was cleaved at its unique BamHI site (Fig. 1), located on the 3' side of LCB1, and the linear DNA was used to transform strain 24D5 with selection for Ura" transformants. Integration at the expected chromosomal location was verified by southern blotting. Transformants were crossed to strain YPH1.

EXAMPLE 4

Plasmids carrying all or portions of LCB2 (Fig. 2) were constructed using standard molecular cloning techniques as follows. Insert B7 is a 7 kb BamHI S. cerevisiae DNA fragment cloned into the BamHI site of pRS315 (Sikorski and Hieter, 1989). Insert B7AS is a 4.9 kb BamHI-Sall fragment cloned into pRS315 at the BamHI-Sall sites of the polylinker. Insert 2.3 is a 2.3 kb BamHI-Sall fragment cloned into pRS316 (Sikorski and Hieter, 1989) at the BamHI-Sacl sites of the polylinker. Insert LCB2-R is a 4.3-kb EcoRI fragment made blunt-ended by filling in the ends with the Klenow fragment of DNA polymerase I and ligated into the SmaI site of pRS315.

EXAMPLE 5

S. cerevisiae strain LCB25, carrying the leb2A3::URA3 allele (Fig. 5), was derived from strain YPH501 as follows. The LCB2-R insert, carried in pC20R, Marsh, J. L., Erle, M. and Wykes, E. J., 1984, Gene 32:481-485, at the EcoRI site of the polylinker, was cleaved with the restriction endonucleases CiaI and XbaI (Fig. 5), the ends of the molecules were made blunt by treatment with the Klenow fragment of DNA polymerase I, and the fragment was ligated to a 1.1 kb URA3 fragment having blunt ends to give the leb2A3::URA3 allele (Fig. 5).

EXAMPLE 6

The integrating vector pRSLCB2-2 (Fig. 5) was constructed by cloning a 2.6- kb BamHI-Apal fragment from the B7 insert into the BamHI-Apal region of the polylinker in pRS305 (Sikorski and Hieter, 1989). pRS305 carries the LEU2 marker gene that was used for selection of transformants in S. cerevisiae strain BS238.

DETAILED DESCRIPTION OF THE FIGURES

FIG. 1

Structure of Plasmids. The plasmid pLCB carrying the LCB1 gene is shown. The approximate location of LCB1 is indicated. Not all restriction endonuclease sites are indicated in a given plasmid. The open arrowhead in pTZ18-LCB1 represents the T7 promoter. DNA sequences are: open box, S. cerevisiae; TRP1, a marker gene for selection in S. cerevisiae; ARS1, a S. cerevisiae autonomous replication sequence; CEN3 a centromere for maintenance of a single-copy of the vector in yeast; BLA and TET confer ampicillin and tetracycline resistance in E. coli, respectively. Abbreviations for restriction endonucleases are: B, BamHI; C, Clal; E, EcorI; H, HindIII; Ha, Hpal; K, Kpnl; P, PstI; S, Sall; Sa, Sau3A; Sac, SacI; Sm, Smal; St, Stul; X, XbaI.

FIG. 2

DNA sequence of LCB1. The nucleotide sequence of the LCB1 gene of S. cerevisiae is presented along with the deduced protein sequence of the 538 amino acids. The predicted translation start codon is indicated by +1.

FIG. 3

Comparison of the deduced amino acid sequence of LCB1 to other proteins. The protein sequences of LCB1 and the mouse (ALSM ((SEQ. ID. NO.:10)), chicken (ALSC ((SEQ ID. NO.:11)), and yeast (ALSY ((SEQ ID. NO.:12)) 5-ami-noeuvulinic acid synthases were compared using the procedures of Pearson and Lipman (1988) and aligned for maximum similarity. The 2-amino-3-ketobutyrate CoA ligase (EKBO ((SEQ ID. NO.:8)) and the biotin synthetase (EBIO ((SEQ ID. NO.:7)) sequences were identified and aligned by using the FASTA algorithm (ibid). Colons (:) represent identity between residues while dots (.) denote conservative replacements by similar residues. Insertions made during the alignment optimization process are indicated by dashes (—).

FIG. 4

Comparison of the reactions catalyzed by serine palmitoyltransferase, ALA synthase, and 2-amino-3-ketobutyrate CoA ligase.

FIG. 5

Structure of Plasmids. A restriction map of the 7 kb BamHI fragment carrying the LCB2 gene is shown at the top of the figure and the approximate location of LCB2 and the direction of transcription are indicated. Not all of the cutting sites for a particular restriction endonuclease are indicated. A. Portions of the region carrying LCB2 were tested for their ability to complement the leb" phenotype of an lecb- defective strain. B. Structure of a deletion allele. C. Structure of the chromosomal insert carried in pRSLCB2-2. Vector sequences are not shown. Abbreviations for restriction endonucleases are: A, Apal; B, BamHI; C, Clal; E, EcorI; Ns, Nsal; Sa, Sall; S, SacI; Sn, SnaBI; Sp, Spal; X, XbaI.

FIG. 6

DNA sequence of LCB2. The nucleotide sequence of the LCB2 gene of S. cerevisiae is presented along with the deduced protein sequence of the 561 amino acids. Numbers on the right side of the figure indicate amino acid residues while numbers on the left indicate nucleotides. The A of the predicted ATG initiation codon has been designated as +1.

FIG. 7

Comparison of the predicted LCB1 ((SEQ ID. NO.:13) and LCB2 ((SEQ ID. NO.:14) protein sequences with each other (identical residues indicated by an asterisk above the sequence) and with the ALA synthase of S. cerevisiae (HEMI$Yeast ((SEQ ID. NO.:15)). Asterisks below the sequence indicate amino acids that are identical in all three sequences while dots (.) indicate amino acids that are similar in the three sequences. Dashes (—) indicate gaps in the sequence introduced to improve alignment.
The invention now being fully described it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth therein. The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without departing from the generic concept and therefore such adaptations are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description only and not of limitation.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 15

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 333
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) MOLECULE TYPE: polynucleotide

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGGCTATTTT TTTTTTTTTG AGGCCGCATG ATTTTCTACA CGGTGTTCTT TTGTTTTCTT 60
TCTTTTCTTC TGTTTTCTCT CTGAAACAAAT TTTTCACTCA TTCTTTTTTA TACGCTGATA 120
TTGCTGCGCT GGAAGTTTAA GAGAATTGGA AAATATTAGT CTTAAAGAAAA 180
AGAAAAAGGAA ATATATAAAA ATATTTTTTT CACACCACTC AGTACCCAG AGTACCG 240
CTCTAAACCT CTGCCCTGCCC TCCAATATAC AACATTTTGC CATGGTAGGGT TATTTATCTT 300
TTTTTTTCCC TCCACCCCA AAAAAAAAAA GCA 333

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1674
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) MOLECULE TYPE: polypeptide

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATG GCA CAC ATC CCA GAG GTT TTA CCC AAA TCA ATA CCG ATT CCG GCA 48
MET Ala His Ile Pro Glu Val Leu Pro Lys Ser Ile Pro Ile Pro Ala 5 15

TTT ATT GGT ACC ACC TCA TCG TAC CTA TGG TAC TAC TTC AAT CTG GTC 96
Pho Ile Val Thr Thr Ser Ser Tyr Leu Trp Tyr Phe Asn Leu Val 20 30

TTG ACT CAA ATC CCG GGA GGC CAA TTC ATC GTT TCG TAC ATC AAG AAA 144
Leu Thr Glu Ile Pro Gly Gly Pro Ala Tyr Ser Tyr Lys Lys 40 50

TCG CAT CAT GAC GAT CCA TAC AGG ACC ACG GTT GAG ATA GGG CCT ATT 192
Ser His His Asp Asp Pro Pro Tyr Arg Thr Thr Val Glu Ile Gly Leu ile 56 75

TTA TAC GGG ATC ATC TAT TAC TGT TCC AAG CCA CAA CAG AAA AAG AGT 240
Leu Tyr Gly Ile Ile Tyr Leu Ser Lys Pro Glu Glu Lys Lys Ser 70 80

CTT CAA GCA GAG AAC CAA TCA TCG CCC CAG GAG ATT GAC GGC CTA 288
Leu Gly Ala Gly Lys Pro Asn Leu Ser Pro Glu Glu Ile Asp Ala Leu 85 95

ATT GAG GAC TGG GAG CCC GAG CCT CTA GTC GAC CTC TCT GCC ACC GAT 336
Ile Gly Asp Trp Glu Pro Glu Pro Leu Asp Pro Ser Ala Thr Asp
<table>
<thead>
<tr>
<th>100</th>
<th>105</th>
<th>110</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAG CAA TCG TGG AOG GTO GCC AAA ACA CCC GTC ACC ATG GAA ATG CCC</td>
<td>384</td>
<td></td>
</tr>
<tr>
<td>ATT CAG AAC CAT ATT ACT ATC ACC AGA AAC AAC CAG AGG GAT TAT Ile Glu Asn His Ile Thr Ile Thr Arg Asn Asn Leu Gln Glu Lys Tyr</td>
<td>432</td>
<td></td>
</tr>
<tr>
<td>ACC AAT GTT AAC AAT TGT TGG GCC TCG AAC AAT TTT TGG CAA TGG TCC GCT</td>
<td>480</td>
<td></td>
</tr>
<tr>
<td>Thr Asn Val Phe Asn Ala Ser Asn Phe Leu Gln Gln Leu Ser Ala</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>ACG GAG CCC GTG AAA GAA GTG GTC AAG ACC ACT ATC AAG AAT TAT GCT</td>
<td>528</td>
<td></td>
</tr>
<tr>
<td>Thr Glu Pro Val Lys Glu Val Val Lys Thr Thr Ile Lys Asn Tyr Gly</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>GTG GCC GCC TGT GGT CCC GCC GGG TTC TAC GGT AAC CAG GAC GTT CAT</td>
<td>576</td>
<td></td>
</tr>
<tr>
<td>Val Gly Ala Cys Gly Pro Ala Gly Pro Gly Lys Asn His</td>
<td>185</td>
<td></td>
</tr>
<tr>
<td>TAC ACG TTT GAA TAT GAT TTA ACA CAG TTC TTT GCC ACC CAA GTT CCA</td>
<td>624</td>
<td></td>
</tr>
<tr>
<td>Tyr Thr Leu Glu Tyr Asp Leu Ala Glu Phe Phe Gly Thr Gly Gln Ser</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>GTC CTC TAC GGT CCC ACA GAC TTT TTT GTG GCC GCA CCC TCT GTG CTG CTC GCT</td>
<td>672</td>
<td></td>
</tr>
<tr>
<td>Val Leu Tyr Gly Glu Asp Phe Cys Ala Ala Pro Ser Gly Val Ser Leu</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>TTC ACA AAG CTG GGT GAT GTT ATC GTG GCA GAC GAC CAG GTG TCA TTA</td>
<td>720</td>
<td></td>
</tr>
<tr>
<td>Phe Thr Lys Arg Arg Asp Val Ile Val Ala Asp Asp Gln Val Ser Leu</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>CCA GTG CAA AAT CTT AAT CTA AAT CAG AAC AGA TTG TAT ACA CTA</td>
<td>768</td>
<td></td>
</tr>
<tr>
<td>Pro Val Glu Asn Ala Leu Gln Leu Ser Ser Arg Thr Tyr Phe</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>AAC CAC AAC GAT AGT AAT TCG CTA GAA TGT TTA TTA AAC GAG TTG ACC</td>
<td>816</td>
<td></td>
</tr>
<tr>
<td>Asp His Asn Asp Met Asn Ser Leu Glu Cys Leu Leu Asn Gln Thr</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>GAA CAG GAG AAA CTT GAG AAA CTT CCC GCC ATT CCA AGA AAA TTT ATC</td>
<td>864</td>
<td></td>
</tr>
<tr>
<td>Glu Glu Glu Lys Leu Gln Leu Pro Ala Ile Pro Arg Lys Phe Ile</td>
<td>255</td>
<td></td>
</tr>
<tr>
<td>GTC ACT GAG GGT ATT TTC CAC AAC TCG GCC GAT TTA GCT CCG TTG CTT</td>
<td>912</td>
<td></td>
</tr>
<tr>
<td>Val Thr Glu Gly Ile Phe His Asn Ser Gly Asp Leu Ala Pro Leu Pro</td>
<td>270</td>
<td></td>
</tr>
<tr>
<td>GAG TGG ACT AAG CTT AAG AAC AAG TAC AAC TAC TTA ACA CTA TTA GGC</td>
<td>960</td>
<td></td>
</tr>
<tr>
<td>Glu Leu Thr Lys Leu Asn Lys Tyr Lys Phe Arg Leu Phe Val Asp</td>
<td>305</td>
<td></td>
</tr>
<tr>
<td>GAA ACC TTC TCC ATT GGT CTG CTT GCC ACG GCC CGT GGG TCA</td>
<td>1008</td>
<td></td>
</tr>
<tr>
<td>Glu Thr Phe Ser Ile Glu Val Gly Ala Thr Arg Gly Lys Gly Ser</td>
<td>325</td>
<td></td>
</tr>
<tr>
<td>GAG CAC TTC AAC ATG GAT GCC GCC ACT GCC ATT GAC ATG CTT GGA</td>
<td>1056</td>
<td></td>
</tr>
<tr>
<td>Glu His Phe Asp Met Asp Arg Ala Thr Ala Ile Asp Ile Thr Val Gly</td>
<td>340</td>
<td></td>
</tr>
<tr>
<td>TCC ATG GCC ACC GGC TGG GCC ACC GGT GGT TTT GTC CCG GTT GAC</td>
<td>1104</td>
<td></td>
</tr>
<tr>
<td>Ser Met Ala Thr Ala Leu Gly Cys Ala Thr Gly Asp</td>
<td>365</td>
<td></td>
</tr>
<tr>
<td>AGT GTT ATG TTT TTC CAC CAG CTT ATT GGT TCC AAC AAT GCA TAT TTC</td>
<td>1152</td>
<td></td>
</tr>
<tr>
<td>Ser Val Met Cys Leu His Gln Arg Ile Gly Ser Asn Ala Tyr Cys Phe</td>
<td>370</td>
<td></td>
</tr>
<tr>
<td>TCC GTC TGG CCG CCT TAC ACC GTC ACA TCC GTC TTC AAA GTC TCC</td>
<td>1200</td>
<td></td>
</tr>
<tr>
<td>Ser Ala Cys Leu Pro Ala Tyr Thr Val Thr Ser Val Ser Lys Val Leu</td>
<td>385</td>
<td></td>
</tr>
<tr>
<td>AAA TGG ATG GCC ACC AAC AGC GCC GTG CAG AAC AGC TCT CAA AAA CTTA</td>
<td>1248</td>
<td></td>
</tr>
<tr>
<td>Lys Leu Met Asp Ser Asn Asp Asn Phe Leu Gln Thr Leu Gln Lys Leu</td>
<td>405</td>
<td></td>
</tr>
<tr>
<td>TCC AAA TCT TGT GAT TCC TTT GTA TGT GAC GTC TGT GAT TCC TCA</td>
<td>1296</td>
<td></td>
</tr>
<tr>
<td>Ser Lys Ser Leu His Asp Phe Ala Ser Asp Asp Ser Leu Arg Ser</td>
<td>420</td>
<td></td>
</tr>
</tbody>
</table>
TAC GTA ATC GTC AGC TCC TCT CCA GTG TCT CCT GTC CTA CAT CTG CAA
Tyr Val Ile Val Thr Ser Ser Pro Val Ser Pro Val Leu His Leu Gin
435 440
CTG ACT CCC GCA TAT AGG TCT CGC AAG TTC GGA TAC ACC TGC GAA CAG
Leu Thr Pro Ala Tyr Arg Ser Arg Lys Phe Gly Tyr Thr Cys Gin Glu
450 460
CTA TTC GAA ACC ATG TCA CTT TGT CAA AAG AAG TCC CAG ACA AAC AAA
Leu Phe Gin Thr Met Ser Ala Leu Gin Lys Ser Ser Gin Thr Asn Lys
465 475 480
TTC ATT GAG CCA TAC GAA GAG GAG GAA AAA TTT CTG CAG TCC ATA GTA
Phe Ile Gin Pro Tyr Glu Gin Glu Gin Lys Phe Leu Gin Ser Ile Val
485 490 495
GAT CAT GCT CTT ATT AAC TAC AAC GTT TCT ATC ACA AGA AAC ACT ATT
Asp His Ala Leu Ile Asn Tyr Asn Val Leu Ile Thr Arg Asn Thr Ile
500 510
GTT TTA AAA CAG GAG ACG CTA CCA ATT GTT TGC CCA AAG TGG AAA ATC TGC
Val Leu Lys Gin Gin Gin Thr Leu Pro Ile Val Pro Ser Leu Lys Ile Cys
520 525
TGT AAC GCC GCT ATG TCC CCA GAG GAA CTC AAA AAT GCT TGC GAA AGT
Cys Asn Ala Ala Met Pro Gin Gin Ala Gin Cys Gin Ser
530 540
GTC AAG CAG TCC ATC CTT GCC TGT TGC CAA GAA TCT AAT AAA
Val Lys Gin Ser Ile Leu Ala Cys Gin Gin Ser Asn Lys
550 555

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 463
   (B) TYPE: nucleic acid
   (C) STRAIGHTNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polynucleotide

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TAAAAATAG AAAGCCAGT ATGCACACGC ACATATATAT ATAAATATTAT ATACAATAAT 60
ACAAATATATC GTGAACTCAT CTCGTCGCAA TTACGCTGCGT GACGCGCGCC CAGAGAAGCG
120
GCTAAAAATT TTTGGAATCC AAATTTTCTT TTTTTTATTCA TATCGAGGCAA AGCAACCTGT 180
ATATATTATT GTTATTATAA TTATAGAAAA AAGAAAGGAT ACTTTTCTTGTC TACGCTTTCTT 240
TGGACATTAT CGGTTTACT CGGCCAGGAA CTAAACACAG AGACACAGCA AACCATCAAC 300
AAGGTTAAAA ACGCAACACA AGGCAATATG ATACATTTTA GAAAAAATAA CAGATACGTT 360
AACACAGGAT ATCAGTACGG AGGAAACCGT GCCGTACAGT TCAAGATTTG TGAAGATGAC 420
AAGGCAAGAT TGAAGATCGG TACTGCTTCC GTGCTGATGC CTA
463

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 884
   (B) TYPE: nucleic acid
   (C) STRAIGHTNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polynucleotide

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCAAAATTTG ATTCTCGATTG AGGCATTTTTC TGGAATTTGAG GTGAAACCTTA TGATGCCTTG
60
TCATGAATT TTAGAGGAGT GCCCGAGAAC AGGCTCTGTTA GTGCGCACCG TTGCCGAAGA
120
GACACCCAAA TGTGCCCATTT ACTTAAAGGCT CATATACTCCT CATA2CTCCT GGAAGATTAG AAATAGGGCA 180
TATGGAAAAT TAAAGTTTTC TGTGTTGTGG CAGCAAGAGA CAGAACCTCG ATATAATGAC 240
ATACGTATAT AATACTACAT GTACAATAATA AGCTACCGAA ATACGTATAA TCTGT2ATAT 300
TACAAAACAA TTACTTCTAT ATCATAAGCCA GTTAAGGGGA AGCAGCTTAC CTAATAAGGC 360
TATCCCATGCT TTAAGGGCAAG GGAAGCCCGG GTGAAGGTGGT GTAACACATC ATCTCTTTCT 420
CTACGACAAA TTTCCAAAAA AAAAAATTTAT GCTATGTATAA TACCTGGACA ATTCACCGCT 480
GCTGAAACGTT AAAAAATGAG TGATTTAAAGC GATAGTATAC GATATTATCA ATCTCATATA 540
AAAAAATCTCT TTGGAATTTA ACGGAAGGAT TATTCATTAG AAAAGGTTTCT TACCATCCCAC 600
TAAAGGACAA TCACTCAGTC CCCTGATATTT CATTACTATT TTTCTATTAT ATCTGCAACT 660
TTTTATTTAG TAGTTTTTTC TTGTTTTTTT GTTTGTTTTCC AATGGAATAA TTTACAGGAC 720
AAAAAAGTCTT ATATTCTGCT GGGGTTGATTA TAAAGGAAAAA AAGACATAAA GAAGATCCCA 780
CACAATTTTAT GGTGATATTT TTTCAAGTAA AAGATTAATAAT ATTA 840

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1683
(B) TYPE: nucleic acid
(C) STRAND:ness: single
(D) TOPOLOGY: linear
(iii) MOLECULE TYPE: peptide

(ii) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TGA GTA CTC CTC CAA ACT ATA CCC TGT GTC CCC TGT GCG AAC CAG ACG
Met Ser Thr Pro Ala Asn Tyr Thr Arg Val Pro Leu Cys Glu Pro Glu 5
10
15
AGC TGC CAG CAG ACA TAC AAA AAG AAA ATG AAT ATG GTA CAC TAG ATT
Glu Leu Pro Asp Asp Ile Glu Lys Glu Asn Glu Tyr Gly Thr Leu Asp 20
25
30
CTC CGG GCC ATT TGT ATC AAG TCA AGT CAC GTC ATG GGA AGC CAC TAC
Ser Pro Gly His Leu Tyr Gln Val Lys Ser Arg His Gly Lys Pro Leu 35
40
45
CTG AGC CCG TTG TCG ACA CCC CTC CTT ATT ACA TTT CTI TGT TAA CAT
Pro Glu Pro Val Thr Asp Pro Thr Val Asp Ser Leu Leu Thr Thr 50
55
60
ATC TAA ATT ATT TGA TTT GTC TAG CAC ATG TTC AAC TCA AGT CAC TGC TAC
Tyr Leu Asn Try Leu Ile Leu Ile Leu Gly His Val His Asp Phe 65
70
75
80
TAG GTA TGC CCT CTC AAA AAA ACA AAC ATC TGG ATC TTT TAG AGC ATG
Leu Gly Met Thr Phe Glu Lys His Leu Asp Leu Gly His Leu Gly 90
95
100
ATG GTG TAG CAC CTT GGT TTT CAA ATT TCG AGA GTC TTT ATG TCA GGA
Asp Gly Leu Ala Pro Thr Phe Ser Asn Phe Glu Ser Phe Tyr Val Arg 105
110
115
GAA TTA AAA TGA GAA TTG ATT GCT TTT CTA GAC CAA CTA CTC GTG
Arg Ile Lys Met Arg Ile Asp Asp Cys Phe Ser Arg Pro Thr Thr Gly 120
125
130
TTC CTT GTA GAT TTA TTC GTT GTA ATA GAA TTT CTC ATA ATA TAA
Val Pro Gly Arg Phe Ile Arg Cys Ile Ser His Asn Ile 135
140
145
150
155
ATG AGT ATT TTA CCT ACT CAG CAG CAG TGT ATC CAT GCA TGA ACT TAT
Asn Glu Tyr Phe Thr Tyr Ser Gly Ala Val Tyr Pro Cys Met Asn Leu 160
165
170
175
180
CAT CAT ATA ACT ATT TAG GCT TCG CAC AAA GTA AGG GTC AAT GTA CGG

5,583,030
Val Val Val Ala Tyr Pro Ala Thr Pro Leu Ile Glu Ser Arg Val Arg
485 490
TCT GTA TGT CTG CAT CTT TAA CAA AGG AAG ATA TCG ATT ATT TAC TGC
500 505
GTC ATG TTA GTG AAG TTG GTG ACA AAT TGA ATT TGA AAT CAA ATT CCG
520 525
GCA AAT CCA GTT AGC ACG GTA AAC GTC AAA GAT GGG ACA TCG AGG AAG
530 535
Gly Lys Ser Ser Tyr Asp Gly Lys Arg Gin Arg Trp Asp Ile Gin Glu
540
TTA TCA GGA GAA CAC CTG AAG ATT GTA AGG ACG ACA AGT ATT TTG TTA
550 555
Val Ile Arg Arg Thr Pro Gin Asp Cys Lys Asp Lys Tyr Phe Val
560
ATT
Asn
1683

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 313
   (B) TYPE: nucleic acid
   (C) STRANDINESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polynucleotide

(x) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GAATTTCCTTAAATGGCTAGTTAGGTTAATCACAAAATTTCTGGAAGACGTTGGAAA60
CAGGCAAGCTTTTGGACATACCTTTAAAACTGGCAAAAATCAACAAAATTTGCAAAA120
AAGGTTAAAAAAAGTTTACGAAAAAAACAAAATTTAAAGAAGAAAGTTAAGAAGTTAC180
GCAATTACGGTCAAGGTATACCAATGAAATAACCTTCTTTTCCACCTTATATAAA240
GTTATATATACAACTTTATCCTATAGTTTACGGTACAAATTACAAAAATTTCTGGAAG300
TTTCGCGACAAAG313

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 49
   (B) TYPE: amino acid
   (C) STRANDINESS: single
   (D) TOPOLOGY: linear

(x) MOLECULE TYPE: polypeptide

(x) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Gln Met Val Val Thr Glu Gly Val Phe Ser Met Asp Gly Asp Ser Ala
5 10 15
Pro Leu Ala Glu Ile Gln Gln Val Thr Gin Gin His Asn Gly Trp Leu
20 25 30
Met Val Asp Ala His Gly Thr Gin Val Ile Gly Glu Gin Gly Arg
35 40 45
Gly

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 132
   (B) TYPE: amino acid
   (C) STRANDINESS: single
   (D) TOPOLOGY: linear
**Molecule Type:** Polypeptide  

**Sequence Description:** Seq ID NO: 5,583,030

<table>
<thead>
<tr>
<th>Phe</th>
<th>Ile</th>
<th>Cys</th>
<th>Gly</th>
<th>Thr</th>
<th>Gln</th>
<th>Leu</th>
<th>Asp</th>
<th>Ser</th>
<th>His</th>
<th>Lys</th>
<th>Glu</th>
<th>Leu</th>
<th>Glu</th>
<th>Gln</th>
<th>Lys</th>
<th>Leu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ala</th>
<th>Ala</th>
<th>Phe</th>
<th>Leu</th>
<th>Gly</th>
<th>Met</th>
<th>Gln</th>
<th>Asp</th>
<th>Ala</th>
<th>Ile</th>
<th>Leu</th>
<th>Tyr</th>
<th>Ser</th>
<th>Ser</th>
<th>Cys</th>
<th>Phe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Asp</th>
<th>Ala</th>
<th>Asn</th>
<th>Gly</th>
<th>Gln</th>
<th>Leu</th>
<th>Phe</th>
<th>Glu</th>
<th>Thr</th>
<th>Leu</th>
<th>Gly</th>
<th>Xaa</th>
<th>Xaa</th>
<th>Ala</th>
<th>Glu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>35</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Asp</th>
<th>Ala</th>
<th>Ile</th>
<th>Ile</th>
<th>Ser</th>
<th>Asp</th>
<th>Ala</th>
<th>Leu</th>
<th>Asn</th>
<th>His</th>
<th>Ala</th>
<th>Ser</th>
<th>Ile</th>
<th>Ile</th>
<th>Asp</th>
<th>Gly</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Val</th>
<th>Arg</th>
<th>Leu</th>
<th>Cys</th>
<th>Lys</th>
<th>Ala</th>
<th>Lys</th>
<th>Arg</th>
<th>Tyr</th>
<th>Arg</th>
<th>Tyr</th>
<th>Ala</th>
<th>Asn</th>
<th>Asn</th>
<th>Asp</th>
<th>Met</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>65</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gin</th>
<th>Glu</th>
<th>Leu</th>
<th>Gla</th>
<th>Arg</th>
<th>Leu</th>
<th>Lys</th>
<th>Gin</th>
<th>Ala</th>
<th>Glu</th>
<th>Arg</th>
<th>Gin</th>
<th>Arg</th>
<th>Gin</th>
<th>Leu</th>
<th>Xaa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>85</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Xaa</th>
<th>Xaa</th>
<th>Xaa</th>
<th>Xaa</th>
<th>Ala</th>
<th>Arg</th>
<th>His</th>
<th>Xaa</th>
<th>Val</th>
<th>Leu</th>
<th>Ile</th>
<th>Ala</th>
<th>Thr</th>
<th>Asp</th>
<th>Gly</th>
<th>Leu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phe</th>
<th>Ser</th>
<th>Met</th>
<th>Asp</th>
<th>Gly</th>
<th>Val</th>
<th>Ile</th>
<th>Ala</th>
<th>Asn</th>
<th>Leu</th>
<th>Lys</th>
<th>Gly</th>
<th>Val</th>
<th>Cys</th>
<th>Asp</th>
<th>Leu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>115</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ala</th>
<th>Asp</th>
<th>Lys</th>
<th>Tyr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>130</td>
</tr>
</tbody>
</table>

(2) Information for Seq ID NO: 5,583,030

**Sequence Characteristics:**

- **Length:** 287 amino acids
- **Type:** Single-stranded, linear
| Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa |
|-----------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| 195                        | 200           | 205           |
| Xaa Xaa Ser Glu His Xaa Xaa Xaa Phe Asn Met Asp Arg Ala Thr Ala Ile |
| 210                        |               |               |
| Asp Ile Thr Val Gly Ser Met Ala Thr Ala Leu Gly Ser Thr Gly Gyl |
| 225                        | 230           | 235           |
| Phe Val Leu Gly Asp Ser Val Met Cys Leu His Gln Arg Ile Gly Ser |
| 245                        | 250           |
| Asn Ala Tyr Cys Phe Ser Ala Cys Leu Pro Ala Tyr Thr Val Thr Ser |
| 260                        | 265           |
| Val Ser Lys Val Leu Lys Leu Met Asp Ser Asn Asn Asp Ala Val |
| 275                        | 280           | 285           |

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 387
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Try Cys Ser Asn Asp Tyr Leu Gly Ile Ser Arg His Pro Arg Val Leu 5   10
Gln Ala Ile Gly Glu Thr Leu Lys Asn His Gly Ala Gly Ala Gly Gly 20  25
Thr Arg Asn Ile Ser Gly Thr Ser Lys Phe His Val Glu Leu Gly Gln 35  40
Glu Leu Ala Gly Leu His Gln Lys Asp Ser Ala Leu Leu Phe Ser Ser 50  55
Cys Phe Val Ala Asn Asp Ser Thr Leu Phe Thr Leu Ala Lys Leu Leu 65  70
Pro Gly Cys Gly Ile Tyr Ser Asp Ala Gly Asn His Ala Ser Met Ile 80  85
Gln Gly Ile Arg Asn Ser Gly Ala Ala Lys Phe Val Phe Arg His Asn 100 105
Asp Pro Gly His Leu Lys Lys Leu Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa 115 120 125
Xaa Xaa Glu Lys Ser Asp Pro Lys Thr Pro Lys Ile Val Ala Phe Glu 130 135 140
Thr Val His Ser Met Asp Gly Ala Ile Cys Pro Leu Glu Glu Leu Cys 145 150 155 160
Asp Val Ala His Gln Tyr Gly Ala Leu Thr Phe Val Asp Glu Val His 165 170 175
Ala Val Gly Leu Tyr Gly Ala Arg Gly Ala Gly Ile Xaa Xaa Xaa Xaa 180 185 190
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa 195 200 205
Xaa Xaa Gly Glu Arg Xaa Xaa Xaa Xaa Asp Gly Ile Met His Lys Leu 210 215 220
Asp Ile Ile Ser Gly Thr Leu Gly Lys Ala Phe Gly Cys Val Gly Gyl 225 230 235 240
Tyr Ile Ala Ser Thr Arg Asp Leu Val Asp Met Val Arg Ser Tyr Ala 245 250 255
Ala Gly Phe Ile Phe Thr Thr Ser Leu Pro Pro Met Met Leu Ser Gly
<table>
<thead>
<tr>
<th>260</th>
<th>265</th>
<th>270</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala Leu Gln Ser Val Arg Leu Leu Lys Gly Gln Gln Gln Ala</td>
<td>275</td>
<td>280</td>
</tr>
</tbody>
</table>

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 287
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:11:

| Trp Cys Ser Asn Asp Tyr Leu Gly Met Ser Arg His Pro Arg Val Cys | 5 | 10 |
| Gly Ala Val Met Asp Thr Lys Leu Gin His Gly Ala Gly Ala Gly Gly | 20 | 30 |
| Thr Arg Asn Ile Ser Gly Thr Ser Lys Phe His Val Asp Leu Gln Lys | 35 | 45 |
| Glu Leu Ala Asp Leu His Gly Lys Asp Ala Ala Leu Leu Phe Ser Ser | 50 | 60 |
| Cys Phe Val Ala Asn Asp Ser Thr Leu Phe Thr Leu Ala Lys Met Leu | 65 | 75 |
| Pro Gly Cys Gin Ile Tyr Ser Asp Ser Gly Asn His Ala Ser Met Ile | 80 | 85 |
| Gin Gly Ile Arg Asn Ser Arg Val Pro Lys His Ile Phe Arg His Asn | 90 | 95 |
| Asp Val Asn His Leu Arg Glu Leu Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa | 100 | 110 |
| Xaa Xaa Lys Lys Ser Asp Pro Ser Thr Pro Lys Ile Val Ala Phe Glu | 115 | 120 | 125 |
| Thr Val His Ser Met Asp Gly Ala Val Cys Pro Leu Gln Gln Leu Cys | 130 | 140 |
| Asp Val Ala His Glu His Gly Ala Ile Thr Phe Val Asp Gln Val His | 145 | 150 | 155 |
| Ala Val Gly Leu Tyr Gly Ala Arg Gly Gly Gly Ile Xaa Xaa Xaa Xaa Xaa | 160 | 165 | 170 | 175 |
| Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa | 180 | 185 | 190 |
| Xaa Xaa Gly Asp Arg Xaa Xaa Xaa Xaa Xaa Gly Val Met His Lys Met | 200 | 205 | 210 | 215 |
| Asp Ile Ile Ser Gly Thr Leu Gly Ala Phe Ala Cys Val Gln Gly Gly | 220 | 225 | 230 | 235 |
| Tyr Ile Ser Ser Thr Ser Ala Leu Ile Asp Thr Val Arg Ser Tyr Ala | 240 | 245 | 250 | 255 |
| Ala Gly Phe Ile Phe Thr Thr Ser Leu Pro Pro Met Leu Leu Ala Gly | 260 | 265 | 270 | 275 |
| Ala Leu Gly Ser Val Arg Thr Leu Lys Ser Ala Glu Gly Gin Val | 280 | 285 | 290 | 295 |

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 287
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(i) MOLECULE TYPE: polypeptide

(ii) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
   Met Ala His Ile Pro Glu Xaa Xaa Xaa Xaa Val Leu Pro Lys Ser Ile
   5  10
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 625
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
   Met Ala His Ile Pro Glu Xaa Xaa Xaa Xaa Val Leu Pro Lys Ser Ile
   5  10
```

Tyr Val Ile Val Thr Ser Ser Pro Val Ser Pro Val Leu His Leu Gin 485
Leu Thr Pro Ala Tyr Arg Ser Arg Lys Phe Gly Xaa Xaa Xaa Xaa Xaa 500
Xaa Xaa Xaa Xaa Tyr Thr Cys Glu Glu Leu Phe Glu Thr Met Ser Ala 510
Leu Glu Lys Lys Ser Glu Thr Asn Lys Phe Ile Gin Pro Tyr Glu Glu 520
Glu Glu Lys Phe Leu Glu Ser Ile Val Asp His Ala Leu Ile Asn Tyr 530
Asn Val Leu Ile Thr Arg Asn Xaa Xaa Xaa Xaa Xaa Thr Ile Val Leu Lys 545
Gln Glu Thr Leu Pro Ile Val Pro Ser Leu Lys Ile Cys Cys Asn Ala 550
Ala Met Ser Pro Glu Glu Leu Lys Asn Ala Xaa Xaa Xaa Xaa Cys Glu Ser 565
Val Lys Gin Ser Ile Leu Ala Cys Cys Gin Glu Ser Asn Xaa Xaa Xaa 580
Lys 590

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 625
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(x) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Ser Thr Pro Ala Asn Tyr Thr Arg Val Pro Leu Cys Glu Pro Glu 5
Glu Leu Pro Asp Asp Ile Gin Lys Glu Asn Glu Tyr Xaa Xaa Xaa Xaa 10
Xaa Xaa Xaa Gly Thr Leu Asp Ser Pro Gly His Leu Tyr Gin Val Xaa 15
Xaa Xaa Xaa Lys Ser Arg His Gly Lys Pro Leu Pro Glu Pro Val Val 20
Asp Thr Pro Pro Tyr Tyr Ile Ser Leu Leu Thr Tyr Leu Asa Tyr Leu 25
Ile Leu Ile Ile Leu Gly His Val His Asp Phe Leu Gly Met Thr Phe 30
Gln Lys Asn Lys His Leu Asp Leu Glu His Asp Gly Leu Ala Pro 35
Trp Phe Ser Asn Phe Glu Ser Phe Tyr Val Arg Arg Ile Lys Met Arg 40
Ile Asp Asp Cys Phe Xaa Xaa Ser Arg Pro Thr Thr Gly Val Pro Gly 45
Arg Phe Xaa Ile Arg Cys Ile Asp Arg Ile Ser His Asn Ile Asn Glu 50
Tyr Phe Thr Tyr Ser Gly Ala Val Tyr Pro Cys Met Asn Leu Ser Ser 55
Tyr Asn Tyr Leu Gly Phe Ala Glu Ser Lys Gly Gin Cys Thr Asp Ala 60

625
<table>
<thead>
<tr>
<th>Ala</th>
<th>Leu</th>
<th>Glu</th>
<th>Ser</th>
<th>Val</th>
<th>Asp</th>
<th>Lys</th>
<th>Tyr</th>
<th>Ser</th>
<th>Ile</th>
<th>Gln</th>
<th>Ser</th>
<th>Gly</th>
<th>Gly</th>
<th>Pro</th>
<th>Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>195</td>
<td></td>
<td>200</td>
<td>204</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>Glu</td>
<td>Ile</td>
<td>Gly</td>
<td>Thr</td>
<td>Thr</td>
<td>Asp</td>
<td>Leu</td>
<td>His</td>
<td>Ile</td>
<td>Lys</td>
<td>Ala</td>
<td>Glu</td>
<td>Lys</td>
<td>Leu</td>
<td>Val</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>210</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>Arg</td>
<td>Phe</td>
<td>Ile</td>
<td>Gly</td>
<td>Lys</td>
<td>Glu</td>
<td>Asp</td>
<td>Ala</td>
<td>Leu</td>
<td>Val</td>
<td>Phe</td>
<td>Ser</td>
<td>Met</td>
<td>Gly</td>
<td>Tyr</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>225</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>Thr</td>
<td>Asn</td>
<td>Ala</td>
<td>Asn</td>
<td>Leu</td>
<td>Phe</td>
<td>Asn</td>
<td>Ala</td>
<td>Phe</td>
<td>Leu</td>
<td>Asp</td>
<td>Lys</td>
<td>Xaa</td>
<td>Lys</td>
<td>Cys</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>245</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Val</td>
<td>Ile</td>
<td>Xaa</td>
<td>Ser</td>
<td>Asp</td>
<td>Glu</td>
<td>Leu</td>
<td>Asn</td>
<td>His</td>
<td>Thr</td>
<td>Ser</td>
<td>Ile</td>
<td>Arg</td>
<td>Thr</td>
<td>Gly</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>260</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>Arg</td>
<td>Leu</td>
<td>Ser</td>
<td>Gly</td>
<td>Ala</td>
<td>Ala</td>
<td>Val</td>
<td>Arg</td>
<td>Thr</td>
<td>Phe</td>
<td>Lys</td>
<td>His</td>
<td>Gly</td>
<td>Asp</td>
<td>Met</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>275</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>Gly</td>
<td>Leu</td>
<td>Glu</td>
<td>Lys</td>
<td>Leu</td>
<td>Ile</td>
<td>Arg</td>
<td>Glu</td>
<td>Gin</td>
<td>Ile</td>
<td>Val</td>
<td>Leu</td>
<td>Gly</td>
<td>Gin</td>
<td>Pro</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>290</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>Thr</td>
<td>Asn</td>
<td>Arg</td>
<td>Pro</td>
<td>Trp</td>
<td>Lys</td>
<td>Ile</td>
<td>Leu</td>
<td>Ile</td>
<td>Cys</td>
<td>Ala</td>
<td>Glu</td>
<td>Gly</td>
<td>Leu</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>310</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>Ser</td>
<td>Met</td>
<td>Glu</td>
<td>Gly</td>
<td>Thr</td>
<td>Leu</td>
<td>Cys</td>
<td>Asn</td>
<td>Leu</td>
<td>Pro</td>
<td>Lys</td>
<td>Leu</td>
<td>Val</td>
<td>Glu</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>335</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>Lys</td>
<td>Lys</td>
<td>Tyr</td>
<td>Lys</td>
<td>Cys</td>
<td>Tyr</td>
<td>Leu</td>
<td>Phe</td>
<td>Ile</td>
<td>Asp</td>
<td>Glu</td>
<td>Ala</td>
<td>His</td>
<td>Ser</td>
<td>Ile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>345</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>Ala</td>
<td>Met</td>
<td>Gly</td>
<td>Pro</td>
<td>Thr</td>
<td>Gly</td>
<td>Arg</td>
<td>Gly</td>
<td>Val</td>
<td>Cys</td>
<td>Gly</td>
<td>Ile</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Phe</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>355</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>Val</td>
<td>Asp</td>
<td>Xaa</td>
<td>Pro</td>
<td>Lys</td>
<td>Asp</td>
<td>Val</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>375</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Asp</td>
<td>Ile</td>
<td>Leu</td>
<td>Met</td>
<td>Gly</td>
<td>Thr</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>385</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>Thr</td>
<td>Lys</td>
<td>Ser</td>
<td>Phe</td>
<td>Gly</td>
<td>Ala</td>
<td>Ala</td>
<td>Gly</td>
<td>Gly</td>
<td>Tyr</td>
<td>Ile</td>
<td>Ala</td>
<td>Ala</td>
<td>Asp</td>
<td>Gin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>405</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trp</td>
<td>Ile</td>
<td>Ile</td>
<td>Asp</td>
<td>Arg</td>
<td>Leu</td>
<td>Arg</td>
<td>Leu</td>
<td>Asp</td>
<td>Leu</td>
<td>Thr</td>
<td>Thr</td>
<td>Val</td>
<td>Ser</td>
<td>Tyr</td>
<td>Ser</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>420</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>Ser</td>
<td>Met</td>
<td>Pro</td>
<td>Ala</td>
<td>Pro</td>
<td>Val</td>
<td>Leu</td>
<td>Ala</td>
<td>Gin</td>
<td>Thr</td>
<td>Ile</td>
<td>Ser</td>
<td>Ser</td>
<td>Leu</td>
<td>Gin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>440</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td>Ile</td>
<td>Ser</td>
<td>Gly</td>
<td>Glu</td>
<td>Ile</td>
<td>Cys</td>
<td>Pro</td>
<td>Gly</td>
<td>Gin</td>
<td>Ile</td>
<td>Cys</td>
<td>Pro</td>
<td>Gly</td>
<td>Gin</td>
<td>Ile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>455</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>Ile</td>
<td>Ala</td>
<td>Phe</td>
<td>Asn</td>
<td>Ser</td>
<td>Arg</td>
<td>Tyr</td>
<td>Leu</td>
<td>Arg</td>
<td>Leu</td>
<td>Ala</td>
<td>Leu</td>
<td>Gin</td>
<td>Arg</td>
<td>Leu</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>470</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>Phe</td>
<td>Ile</td>
<td>Val</td>
<td>Tyr</td>
<td>Glu</td>
<td>Val</td>
<td>Ala</td>
<td>Asp</td>
<td>Ser</td>
<td>Pro</td>
<td>Val</td>
<td>Ile</td>
<td>Pro</td>
<td>Leu</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>485</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Tyr</td>
<td>Cys</td>
<td>Pro</td>
<td>Ser</td>
<td>Lys</td>
<td>Met</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Pro</td>
<td>Ala</td>
<td>Phe</td>
<td>Ser</td>
<td>Arg</td>
<td>Met</td>
<td>Xaa</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>520</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Glu</td>
<td>Arg</td>
<td>Arg</td>
<td>Ile</td>
<td>Ala</td>
<td>Val</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Val</td>
<td>Val</td>
<td>Val</td>
<td>Ala</td>
<td>Tyr</td>
<td>Pro</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>530</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td>Pro</td>
<td>Xaa</td>
<td>Leu</td>
<td>Ile</td>
<td>Glu</td>
<td>Ser</td>
<td>Arg</td>
<td>Val</td>
<td>Arg</td>
<td>Phe</td>
<td>Cys</td>
<td>Met</td>
<td>Ser</td>
<td>Ala</td>
<td>Xaa</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>545</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xaa</td>
<td>Ser</td>
<td>Leu</td>
<td>Thr</td>
<td>Lys</td>
<td>Glu</td>
<td>Asp</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Xaa</td>
<td>Ile</td>
<td>Asp</td>
<td>Tyr</td>
<td>Leu</td>
<td>Leu</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>565</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>His</td>
<td>Val</td>
<td>Ser</td>
<td>Glu</td>
<td>Val</td>
<td>Gly</td>
<td>Asp</td>
<td>Lys</td>
<td>Leu</td>
<td>Asn</td>
<td>Leu</td>
<td>Lys</td>
<td>Ser</td>
<td>Asn</td>
<td>Ser</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>580</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>Lys</td>
<td>Ser</td>
<td>Ser</td>
<td>Tyr</td>
<td>Asp</td>
<td>Gly</td>
<td>Lys</td>
<td>Arg</td>
<td>Gin</td>
<td>Arg</td>
<td>Trp</td>
<td>Asp</td>
<td>Ile</td>
<td>Glu</td>
<td>Glu</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>595</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>Ile</td>
<td>Arg</td>
<td>Arg</td>
<td>Thr</td>
<td>Pro</td>
<td>Glu</td>
<td>Asp</td>
<td>Cys</td>
<td>Lys</td>
<td>Asp</td>
<td>Asp</td>
<td>Lys</td>
<td>Tyr</td>
<td>Phe</td>
<td>Val</td>
</tr>
</tbody>
</table>
(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 635
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(x) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Gln Arg Xaa Ser Ile Phe Ala Arg Xaa Xaa Phe Gly Asn Ser Ser 10 15
Ala Ala Val Ser Thr Leu Asn Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa 20 30
Xaa Xaa Xaa Xaa Xaa Leu Ser Thr Thr Ala Ala Pro His Ala Lys Asn Gly 35 40 45
Tyr Ala Thr Ala Thr Gly Ala Gly Ala Ala Ala Ala Thr Ala Thr Ala 50 55 60
Ser Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa 65 70 75 80
Xaa Xaa Xaa Xaa Xaa Thr His Ala Ala Ala Ala Ala Ala Ala 85 90 95
Asn His Ser Thr Gln Glu Ser Gly Phe Asp Tyr Glu Gly Leu Ile Asp 100 105 110
Xaa Xaa Ser Glu Leu Gln Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Lys Arg 115 120 125
Leu Asp Lys Ser Tyr Arg Tyr Phe Asn Asn Ile Asn Arg Leu Ala Lys 130 135 140
Glu Phe Pro Leu Ala His Arg Gln Arg Glu Ala Asp Lys Val Thr Val 145 150 155 160
Trp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Ser 165 170 175
Asn Asp Tyr Leu Ala Leu Ser Lys Xaa His Pro Gln Val Leu Asp Ala 180 185 190
Met His Lys Thr Ile Asp Lys Tyr Gly Cys Gly Ala Gly Gly Thr Arg 195 200 205
Asn Ile Ala Gly His Asn Ile Pro Thr Leu Asn Leu Glu Ala Glu Leu 210 215 220
Ala Thr Leu His Lys Gly Ala Leu Val Phe Ser Ser Cys Tyr 225 230 235 240
Val Ala Asn Asp Ala Val Leu Ser Leu Glu Gly Gin Lys Met Lys Asp 245 250 255
Leu Val Ile Phe Ser Asp Glu Leu Asn His Ala Ser Met Ile Val Gly 260 265 270
Ile Lys His Ala Asn Val Lys His Ile Phe Lys His Asn Asp Leu 275 280 285
Asn Glu Leu Glu Gin Leu Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa 290 295 300
Gln Ser Tyr Pro Lys Ser Val Pro Lys Leu Ile Ala Phe Glu Ser Val 305 310 315 320
Tyr Ser Met Ala Gly Ser Val Ala Asp Ile Glu Lys Ile Cys Asp Leu 325 330 335
What is claimed is:
1. A DNA sequence LCB1 having the nucleotide sequence of Sequence ID Nos: 1–3, wherein LCB stands for long chain base.
2. A plasmid comprising the LCB1 sequence according to claim 1.
3. A plasmid according to claim 2, which is the plasmid pTZ18-LCB1.
4. A plasmid according to claim 2, which is YlpLCB1-1.
5. A host cell transformed by a plasmid to comprise and express an LCB1 sequence according to claim 1.
6. A DNA sequence LCB2 having the nucleotide sequence of Sequence ID Nos: 4–6.
7. A plasmid comprising the DNA sequence according to claim 6.
8. A plasmid according to claim 7, which is pRSLCB2-2.
9. A host cell transformed by a plasmid to comprise and express an LCB2 sequence according to claim 6.
10. A genetically engineered microbial strain transformed by a plasmid comprising both the LCB1 and LCB2 sequences of claims 1 and 6, wherein said plasmid overexpresses the genes with which it has transformed and overproduces the Serine Palmitoyltransferase enzyme.
11. A DNA sequence which is a complement to the sequence according to claim 1.
12. A DNA sequence which is a complement to the sequence according to claim 5.

* * * * *