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(54) **SCATTERED BRANCHED-CHAIN FATTY ACIDS AND BIOLOGICAL PRODUCTION THEREOF**

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(57) **ABSTRACT**

Methods and cells for producing scattered branched-chain fatty acids are provided. For example, the invention provides a method for producing branched-chain fatty acid comprising a methyl on one or more even number carbons. The method comprises culturing a cell comprising an exogenous or over-expressed polynucleotide comprising a nucleic acid sequence encoding a polypeptide that catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA and/or an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a polypeptide that catalyzes the conversion of succinyl-CoA to methylmalonyl-CoA, under conditions allowing expression of the polynucleotide(s) and production of branched-chain fatty acid. The cell produces more branched-chain fatty acid comprising a methyl on one or more even number carbons than an otherwise similar cell that does not comprise the polynucleotide(s). A cell that produces branched-chain fatty acid and the branched-chain fatty acid also are provided.

Figure 1: *mutA* nucleotide sequence (SEQ.ID NO: 1)

ATGGCAAGCACGGACCAGGGTACCAACCCGGCAGACACCGACGACCTGACGCCAACCACT
CTGAGTCTGGCGGGCGATTTTCCGAAAGCAACCGAAGAACAGTGGGAGCGCGAAGTGGAG
AAAGTTCTGAACCGTGGCCGTCCGCCGGAGAAACAGCTGACGTTTGCGGAATGTCTGAAA
CGCCTGACGGTCCACACAGTAGACGGCATTGACATTGTGCCAATGTATCGCCCGAAAGAT
GCGCCGAAGAAACTGGGTTACCCAGGCGTTGCCCCATTTACACGTGGGACCACGGTTCGT
AATGGCGATATGGACGCATGGGATGTCCGTGCACTGCATGAAGATCCGGATGAGAAATTT
ACGCGCAAAGCGATTCTGGAAGGGCTGGAACGCGGGGTTACATCTCTGCTGCTGCGTGTG
GACCCGGACGCTATTGCTCCAGAACACCTGGATGAAGTGCTGTCTGACGTGCTGCTGGAG
ATGACCAAAGTAGAAGTCTTTAGTCGTTACGATCAAGGCGCCGCTGCCGAGGCGCTGGTA
TCTGTGTACGAGCGCAGCGATAAACCGGCTAAGGACCTGGCTCTGAATCTGGGTCTGGAC
CCGATCGCCTTCGCGGCACTGCAGGGGACGGAACCTGATCTGACTGTCCTGGGTGATTGG
GTGCGTCGCCTGGCAAAATTTAGCCCAGATTCTCGTGCAGTGACCATCGATGCGAACATT
TATCATAATGCGGGTGCGGGCGATGTAGCAGAGCTGGCTTGGGCCCTGGCTACCGGTGCG
GAATATGTTCGTGCACTGGTAGAACAAGGTTTTACGGCGACCGAGGCGTTCGATACGATT
AACTTTCGTGTGACCGCAACCCATGATCAGTTTCTGACAATCGCGCGTCTGCGCGCACTG
CGTGAGGCGTGGGCGCGCATTGGGGAGGTATTTGGGGTTGATGAGGATAAACGTGGCGCC
CGTCAAAATGCGATCACGAGTTGGCGCGATGTGACACGCGAGGACCCGTATGTGAATATC
CTGCGCGGGAGCATCGCTACATTTTCTGCAAGCGTGGGTGGGGCCGAAAGTATTACAACT
CTGCCTTTTACCCAGGCACTGGGTCTGCCAGAAGACGATTTTCCGCTGCGTATCGCTCGT
AATACCGGTATCGTTCTGGCCGAAGAAGTGAACATCGGTCGTGTTAATGATCCGGCCGGC
GGTAGCTATTACGTGGAAAGTCTGACTCGTAGTCTGGCCGATGCAGCGTGGAAAGAGTTC
CAAGAAGTGGAGAAACTGGGCGGCATGAGCAAGGCGGTGATGACGGAACATGTAACGAAA
GTGCTGGATGCCTGCAATGCAGAACGCGCGAAACGCCTGGCCAATCGCAAACAGCCGATT
ACCGCAGTAAGCGAATTTCCTATGATTGGGGCGCGCTCTATCGAAACGAAACCTTTTCCT
GCCGCACCGGCCCGTAAAGGTCTGGCATGGCATCGCGACAGTGAAGTATTCGAACAACTG
ATGGATCGCAGCACCAGTGTGAGTGAACGTCCAAAGGTTTTCCTGGCGTGCCTGGGCACA
CGTCGTGACTTCGGTGGTCGTGAGGGTTTTAGCAGCCCAGTGTGGCATATCGCAGGCATT
GACACCCCACAGGTTGAGGGTGGCACAACCGCAGAAATCGTAGAAGCATTCAAGAAATCT
GGGGCACAAGTTGCGGATCTGTGCTCTAGCGCCAAAGTGTACGCTCAGCAGGGTCTGGAG
GTGGCCAAAGCTCTGAAAGCAGCTGGCGCCAAAGCCCTGTATCTGAGCGGTGCCTTTAAG
GAGTTCGGCGATGATGCGGCTGAGGCGGAGAAACTGATCGATGGTCGCCTGTTTATGGGT
ATGGATGTGGTTGACACTCTGTCTAGTACGCTGGACATTCTGGGTGTAGCAAAGTAA

Figure 2: *mutB* nucleotide sequence (SEQ.ID NO: 2)

```
ATGACT
ACACTGCCTCGTTTTGACTCTGTTGACCTGGGGAACGCGCCTGTTCCGGCGGATGCGGCC
CGTCGCTTCGAGGAACTGGCGGCAAAAGCGGGCACGGGTGAGGCGTGGGAGACCGCGGAG
CAGATTCCGGTTGGTACACTGTTCAATGAAGACGTTTACAAAGATATGGACTGGCTGGAC
ACGTACGCCGGGATTCCGCCATTCGTTCACGGCCCGTACGCGACGATGTACGCTTTCCGT
CCGTGGACAATTCGTCAATACGCCGGGTTTAGCACGGCGAAAGAAAGTAATGCTTTCTAC
CGCCGTAACCTGGCGGCGGGGCAAAAGGGTCTGTCTGTGGCATTCGACCTGCCGACCCAC
CGCGGTTACGATAGCGATAATCCGCCGCGTGGCAGGGGACGTGGGTATGGCCGGGGTGGCC
ATCGACAGTATTTACGACATGCGTGAACTGTTTGCAGGCATTCCGCTGGACCAGATGAGC
GTGAGTATGACGATGAATGGTGCCGTCCTGCCGATTCTGGCACTGTATGTGGTTACAGCC
GAAGAACAAGGTGTGAAGCCGGAACAGCTGGCTGGCACCATCCAGAACGATATTCTGAAG
GAGTTCATGGTGCGTAACACCTATATCTATCCGCCGCAACCGTCTATGCGCATCATCAGT
GAGATCTTTGCGTATACTAGTGCAAATATGCCGAAGTGGAACTCTATCAGTATTAGTGGC
TATCACATGCAGGAGGCGGGCGCCACTGCCGATATCGAAATGGCCTATACGCTGGCCGAT
GGCGTTGATTATATTCGTGCAGGCGAAAGCGTCGGTCTGAACGTGGACCAGTTCGCCCCG
CGTCTGAGCTTCTTTTGGGGTATTGGCATGAATTTCTTTATGGAAGTCGCAAAACTGCGT
GCCGCCCGCATGCTGTGGGCCAAACTGGTGCACCAATTCGGCCCGAAGAACCCGAAGAGC
ATGAGCCTGCGCACGCACAGTCAAACCAGCGGCTGGAGCCTGACCGCGCAGGACGTATAT
AACAACGTAGTTCGCACCTGTATTGAGGCGATGGCAGCCACCCAGGGTCACACCCAGAGC
CTGCATACAAACTCTCTGGACGAGGCCATCGCACTGCCGACAGACTTCAGCGCCCGCATC
GCGCGTAATACTCAACTGTTTCTGCAACAGGAAAGCGGTACTACCCGTGTGATCGATCCG
TGGTCTGGCAGTGCATATGTCGAGGAACTGACCTGGGATCTGGCCCGTAAAGCGTGGGGT
CATATCCAGGAAGTCGAGAAAGTGGGTGGTATGGCTAAAGCAATTGAGAAAGGCATCCCG
AAAATGCGCATTGAAGAAGCGGCAGCGCGCACCCAAGCACGCATCGACAGCGGTCGCCAG
CCGCTGATTGGCGTGAACAAATATCGCCTGGAACATGAACCGCCACTGGATGTTCTGAAA
GTAGATAACTCTACCGTCCTGGCGGAGCAGAAAGCGAAACTGGTTAAGCTGCGTGCGGAA
CGCGATCCTGAGAAAGTTAAAGCGGCGCTGGATAAAATCACTTGGGCCGCGGGCAACCCG
GATGATAAAGACCCAGACCGTAATCTGCTGAAGCTGTGTATTGACGCGGGTCGTGCTATG
GCGACTGTCGGCGAAATGAGCGATGCGCTGGAGAAAGTATTTGGTCGTTATACCGCGCAA
ATTCGTACTATTTCTGGTGTCTATAGCAAGGAAGTTAAGAATACTCCAGAAGTAGAAGAA
GCGCGTGAACTGGTAGAAGAATTTGAGCAGGCTGAAGGTCGCCGTCCACGCATTCTGCTG
GCCAAAATGGGCCAGGATGGCCATGATCGCGGTCAGAAAGTTATTGCTACTGCTTATGCT
GATCTGGGCTTCGATGTTGATGTCGGCCCTCTGTTCCAGACTCCAGAGGAAACTGCCCGC
CAGGCTGTTGAAGCTGACGTCCATGTCGTTGGCGTTAGCTCTCTGGCTGGCGGCCATCTG
ACCCTGGTCCCTGCTCTGCGCAAGGAACTGGATAAGCTGGGCCGCCCTGATATTCTGATT
ACTGTCGGCGGCGTCATTCCTGAACAGGATTTCGATGAACTGCGCAAGGATGGCGCTGTC
GAAATTTATACCCCTGGCACCGTCATTCCTGAATCTGCTATTTCTCTGGTCAAGAAGCTG
CGCGCTAGCCTGCATGCCTAACTCCAG
```

Figure 3: MutA protein sequence (SEQ.ID NO: 3)

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MARTYAGHSSAAASNALYRRNLAKGQTGLSVAFDLPTQTGYDPDHVLARGEVGKVGVPISHIGDMRALFDQ
TPLGQMNTSMTTNATAMWLLAMYQVAAEDQATAADFDPASVVKALGGTTQNDITKEYLSRGTYVFAPAPS
LRLITDMVSYTVSDIPKWNPINICSYHLQEAGATPVQEIAYAMSTAIAVLDAVRDAGQVPQERFGEVVAR
ISFFVNAGVRFVEEMCKMRAFVELWDELTRERYGVTDAKQRRFRYGVQVNSLGLTEAQPENNVQRIVLEM
LAVTLSKGARARAVQLPAWNEALGLPRPWDQQWSLRMQQVLAYESDLLEYEDLFEGSAVVEAKVAELVAG
AKAEIARVAELGGAVAAVESGYMKSALVASHALRRQRIEAGEDIVVGVNKFETTEPNPLTADLDTAIQSV
DAGVEAAAAKAVREWRETRDADPVKRERAVAALARLKAAAQTDENLMEASIECARAEVTTGEWAQALREV
FGEFRAPTGVTGTVGLTGGAAGAELSAVRERVAGLRDELGETLRVLVGKPGLDGHSNGAEQIAVRARDAG
FEVIYQGIRLTPEQIVAAAVSEDVHLVGISILSGSHMELIPEVLDRLREAGAGDIPVIVGGIIPESDAAK
LKAIGVAEVFTPKDFGLNDIMGRFVDVIRDSRLTTAAPTV
```

Figure 4: MutB protein sequence (SEQ.ID NO: 4)

MTVAPKRPAAMTLAAHFPERTQEQWRDLVACVVNKGRPEDQHLSGDDAVATMRSHLEGGLDIEPLYMKSSDPVPLGVP
GAMPFTRGRALRDADVPWDVRQVHDDPDAAATRQLVLADLENGVTSVWLHVGADGLAPNDVAEALAEVRLELAPVVVS
SWDDQTAAADALYAVLSGSRASSGNLGHDPLGAAARTGSAPDLAPLADAVRRLADHGEIRAITVDTRVHGDAGVTVTD
EVAFALATGVAYLRHLESEGVDVAEAFRNIEFRVSATADQFLTAAALRALRRAWARIGESVGVPETSRGAFTHAVTSG
RIFTRDDAWTNILRSTLATFGASLGGADAITVLPFDTVSGLPTPFSRRIARNTQILLAEESNVARVTDPAGGSWYVET
LTDDVAKAAWETFQEIESAGGMVAALANGLVAQRILAAVAERDAALATRSTPITGVSTFPLAGEKPLERVVRAELPVQ
PNALAPHRDSAIFEALRDRSAAYATEHGHAPRVSVPTLDVPRAADRRIDAVNLLTVAGIDAVDGDTESAAALTGTDKG
YEGVAKDMDVVAFLSDLLDTTGAPA

Figure 5: Methylmalonyl-CoA epimerase nucleotide sequence (SEQ.ID NO: 5)

GAATTCAGGAGGTCCTTCCATATGCTGACCCGCATCGATCACATTGGCATCGCATGCTTTGAT
CTGGATAAAACCGTAGAGTTCTATCGCGCCACCTACGGCTTTGAGGTGTTTCATAGCGAA
GTAAACGAAGAACAGGGCGTGCGTGAAGCCATGCTGAAAATCAACGAAACTAGTGATGGT
GGGGCGAGCTATCTGCAACTGCTGGAACCGACACGCCCGGACTCTACAGTTGCTAAGTGG
CTGGACAAGAATGGCGAAGGCGTTCATCACATTGCGTTCGGTACGGCTGATGTGGATCAA
GACGCGGCAGATATTAAAGATAAGGGTGTGCGTGTTCTGTACGAGGAGCCACGCCGTGGT
AGCATGGGTAGCCGTATTACGTTCCTGCACCCTAAAGACTGTCATGGTGTGCTGACTGAG
CTGGTCACCTCTGCCCCGGTCGAAAGTCCGGAACATTAAAAGCTT

Figure 6: Methylmalonyl-CoA epimerase protein sequence (SEQ.ID NO: 6)
MLTRIDHIATACFDLDKTVEFYRATYGFEVFHSEVNEEQGVREAMLKINETSDGGASYLQLLEPTRPDSTVAKWLDKN

GEGVHHIAFGTADVDQDAADIKDKGVRVLYEEPRRGSMGSRITFLHPKDCHGVLTELVTSAPVESPEH

Figure 7: DNA sequence for *accA1* (AF113603.1) (SEQ.ID NO: 7)

GTGCGCAAGGTGCTCATCGCCAATCGTGGCGAAATCGCTGTCCGCGTGGCCCGGGCCTGCCGGGACGCCG
GGATCGCGAGCGTGGCCGTCTACGCGGATCCGGACCGGGACGCGTTGCACGTCCGTGCCGCTGATGAGGC
GTTCGCCCTGGGTGGTGACACCCCGCGACCAGCTATCTGGACATCGCCAAGGTCCTCAAAGCCGCGCGC
GAGTCGGGCGCGGACGCCATCCACCCCGGGTACGGATTCCTCTCGGAGAACGCCGAGTTCGCGCAGGCGG
TCCTGGACGCCGGCCTGATCTGGATCGGCCCGCCCCCGCACGCCATCCGCGACCGTGGCGAAAAGGTCGC
CGCCCGCCACATCGCCCAGCGGGCCGGCGCCCCCCTGGTCGCCGGCACCCCGACCCCGTCTCCGGCGCG
GACGAGGTCGTCGCCTTCGCCAAGGAGCACGGCCTGCCCATCGCCATCAAGGCCGCCTTCGCGCGGCGGC
GGCGCGGCCTCAAGGTCGCCCGCACCCTCGAAGAGGTGCCGGAGCTGTACGACTCCGCCGTCCGCGAGGC
CGTGGCCGCCTTCGGCCGCGGGGAGTGCTTCGTCGAGCGCTACCTCGACAAGCCCCGCCACGTGGAGACC
CAGTGCCTGGCCGACACCCACGGCAACGTGGTCGTCGTCTCCACCCGCGACTGCTCCCTCCAGCGCCGCC
ACCAAAAGCTCGTCGAGGAGCCCCCCCCCCCTTTCTCTCCGAGGCCCAGACCGACCAGCCTACTCATC
CTCCAAGGCCATCCTGAAGGAGGCCGGCTACGGCGGCGCCGGCACCGTGGAGTTCCTCGTCGGCATGGAC
GGCACGATCTTCTTCCTGGAGGTCAACACCCGCCTCCAGGTCGAGCACCCGGTCACCGAGGAAGTCGCCG
GCATCGACTTGGTCCGCGAGATGTTCCGCATCGCCGACGGCGAGGAACTCGGTTACGACGACCCCGCCCT
GCCCGGCCACTCCTTCGAGTTCCGCATCAACGCCGAGGACCCCGGCCTGGGCTTCCTGCCCGCCCCCGGC
ACCGTCACCCTCTTCGACGCGCCCACCGGCCCCGGCGTCCGCCTGGACGCCGGCGTCGAGTCCGGCTCCG
TCATCGGCCCCGCCTGGGACTCCCTCCTCGCCAAACTGATCGTCACCGGCCGCACCCGCGCCGAGGCACT
CCAGCGCGCGGCCCGCGCCCTGGACGAGTTCACCGTCGAGGGCATGGCCACCGCCATCCCCTTCCACCGC
ACCGTCCTCCGCGACCCGGCCTTCCCCCCCGAACTCACCGGCTCCACGGACCCCTTCACCCTCCACACCC
GGTGGATCGAGACGGAGTTCGTCAACGAGATCAAGCCCTTCACCACGCCCGCCGACACCGAGACGGACGA
GGAGTCGGGCCGGGAGACGGTCGTCGTCGAGGTCGGCGGCAAGCGCCTGGAAGTCTCCCTCCCCTCCAGC
CTGGGCATGTCCCTGGCCCCGCACCGGCCTGGCCGCCGGGGCCCGCCCCAAGCGCCGCGCGCCAAGAAGT
CCCGCCCCGCCGCCCTCCGGCCACACCCTCGCCTCCCCGATGCAGGGCACGATCGTCAAGATCGCCGTCGA
GGAAGGCCAGGAAGTCCAGGAAGGCGACCTCATCGTCGTACTCGAGGTGATGAAGATGGAACAGCCCCTC
AACGCCCACAGGTCCGGCACCATCAAGGGCCTCACCGCCGAGGTCGGCGCCTCCCTCACCTCCGGCGCCG
CCATCTGCGAGATCAAGGACTGA

Figure 8: DNA sequence for *pccB* (AF113605.1) (SEQ.ID NO: 8)

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ATGTCCGAGCCGGAAGAGCAGCAGCCCGACATCCACACGACCGCGGGCAAGCTCGCGGATCTCAGGCGCC
GTATCGAGGAAGCGACGCACGCCGGTTCCGCACGCGCCGTCGAGAAGCAGCACGCCAAGGGCAAGCTGAC
GGCTCGTGAACGCATCGACCTCCTCCTCGACGAGGGTTCCTTCGTCGAGCTGGACGAGTTCGCCCGGCAC
CGCTCCACCAACTTCGGCCTCGACGCCAACCGCCCCTACGGCGACGGCGTCGTCACCGGCTACGGCACCG
TCGACGGCCGCCCCGTGGCCGTCTTCTCCCAGGACTTCACCGTCTTCGGCGGCGCGCTGGGCGAGGTCTA
CGGCCAGAAGATCGTCAAGGTGATGGACTTCGCCCTCAAGACCGGCTGCCCGGTCGTCGGCATCAACGAC
TCCGGCGGCGCCCGCATCCAGGAGGGCGTGGCCTCCCTCGGCGCCTACGGCGAGATCTTCCGCCGCAACA
CCCACGCCTCCGGCGTGATCCCGCAGATCAGCCTGGTCGTCGGCCCGTGTGCGGGCGGCGCGGTGTACTC
CCCCGCGATCACCGACTTCACGGTGATGGTGGACCAGACCAGCCACATGTTCATCACCGGTCCCGACGTC
ATCAAGACGGTCACCGGCGAGGACGTCGGCTTCGAGGAGCTGGGCGGCGCCCGCACCCACAACTCCACCT
CGGGCGTGGCCCACCACATGGCCGGCGACGAGAAGGACGCGGTCGAGTACGTCAAGCAGCTCCTGTCGTA
CCTGCCGTCCAACAACCTCTCCGAGCCCCCCGCCTTCCCGGAGGAGGCGGACCTCGCGGTCACGGACGAG
GACGCCGAGCTGGACACGATCGTCCCGGACTCGGCGAACCAGCCCTACGACATGCACTCCGTCATCGAGC
ACGTCCTGGACGACGCCGAGTTCTTCGAGACGCAACCCCTCTTCGCGCCGAACATCCTCACCGGCTTCGG
CCGCGTGGAGGGCCGCCCGGTCGGCATCGTCGCCAACCAGCCCATGCAGTTCGCCGGCTGCCTGGACATC
ACGGCCTCCGAGAAGGCGGCCCGCTTCGTGCGCACCTGCGACGCCTTCAACGTCCCCGTCCTCACCTTCG
TGGACGTCCCCGGCTTCCTGCCCGGCGTCGACCAGGAGCACGACGGCATCATCCGCCGCGGCGCCAAGCT
GATCTTCGCCTACGCCGAGGCCACGGTGCCGCTCATCACGGTCATCACCCGCAAGGCCTTCGGCGGCGCC
TACGACGTCATGGGCTCCAAGCACCTGGGCGCCGACCTCAACCTGGCCTGGCCCACCGCCCAGATCGCCG
TCATGGGCGCCCAAGGCGCGGTCAACATCCTGCACCGCCGCACCATCGCCGACGCCGGTGACGACGCCGA
GGCCACCCGGGCCCGCCTGATCCAGGAGTACGAGGACGCCCTCCTCAACCCCTACACGGCGGCCGAACGC
GGCTACGTCGACGCCGTGATCATGCCCTCCGACACTCGCCGCCACATCGTCCGCGGCCTGCGCCAGCTGC
GCACCAAGCGCGAGTCCCTGCCCCCGAAGAAGCACGGCAACATCCCCCTGTAA
```

Figure 9: Protein sequence for AccA1 (SEQ.ID NO: 9)

```
MRKVLIANRGEIAVRVARACRDAGIASVAVYADPDRDALHVRAADEAFALGGDTPATSYL
DIAKVLKAARESGADAIHPGYGFLSENAEFAQAVLDAGLIWIGPPPHAIRDRGEKVAARH
IAQRAGAPLVAGTPDPVSGADEVVAFAKEHGLPIAIKAAFGGGGRGLKVARTLEEVPELY
DSAVREAVAAFGRGECFVERYLDKPRHVETQCLADTHGNVVVVSTRDCSLQRRHQKLVEE
APAPFLSEAQTEQLYSSSKAILKEAGYGGAGTVEFLVGMDGTIFFLEVNTRLQVEHPVTE
EVAGIDLVREMFRIADGEELGYDDPALRGHSFEFRINGEDPGRGFLPAPGTVTLFDAPTG
PGVRLDAGVESGSVIGPAWDSLLAKLIVTGRTRAEALQRAARALDEFTVEGMATAIPFHR
TVVRDPAFAPELTGSTDPFTVHTRWIETEFVNEIKPFTTPADTETDEESGRETVVVEVGG
KRLEVSLPSSLGMSLARTGLAAGARPKRRAAKKSGPAASGDTLASPMQGTIVKIAVEEGQ
EVQEGDLIVVLEAMKMEQPLNAHRSGTIKGLTAEVGASLTSGAAICEIKD*
```

Figure 10: Protein sequence for PccB (SEQ.ID NO: 10)

```
MSEPEEQQPDIHTTAGKLADLRRRIEEATHAGSARAVEKQHAKGKLTARERIDLLLDEGS
FVELDEFARHRSTNFGLDANRPYGDGVVTGYGTVDGRPVAVFSQDFTVFGGALGEVYGQK
IVKVMDFALKTGCPVVGINDSGGARIQEGVASLGAYGEIFRRNTHASGVIPQISLVVGPC
AGGAVYSPAITDFTVMVDQTSHMFITGPDVIKTVTGEDVGFEELGGARTHNSTSGVAHHM
AGDEKDAVEYVKQLLSYLPSNNLSEPPAFPEEADLAVTDEDAELDTIVPDSANQPYDMHS
VIEHVLDDAEFFETQPLFAPNILTGFGRVEGRPVGIVANQPMQFAGCLDITASEKAARFV
RTCDAFNVPVLTFVDVPGFLPGVDQEHDGIIRRGAKLIFAYAEATVPLITVITRKAFGGA
YDVMGSKHLGADLNLAWPTAQIAVMGAQGAVNILHRRTIADAGDDAEATRARLIQEYEDA
LLNPYTAAERGYVDAVIMPSDTRRHIVRGLRQLRTKRESLPPKKHGNIPL*
```

Figure 11: Element 1: PlacO1 sequence + phage T7 gene10 ribosome binding site (SEQ.ID NO: 11)

```
aattgtgagcggataacaattgacattgtgagcggataacaagatactgagcacatcagcaggacgcactgaccgaattcaataat
tttgtttaactttaagaaggagatatacat
```

Figure 12: Element 2: Optimized *accA1* gene sequence (SEQ.ID NO: 12)

```
atgcgcaaagtgctgattgcgaaccgtggtgaaatcgccgttcgtgtggcacgcgcgtgtcgtgatgcaggtattgcaagtgttgcggtgtatgccg
atccggatcgcgatgcgctgcatgttcgtgcggccgatgaagcctttgcactgggcggtgataccccggcaacgagctatctggatatcgcaaaagt
gctgaaagcagcgcgcgaaagcggtgcggatgccatccatccgggctacggttttctgtctgaaaatgcagaatttgcacaggcggttctggatgca
ggtctgatttggatcggtccgccgccgcatgcaattcgtgatctgggcgataaagtggccgcacgccacatcgcccagcgtgcaggcgcgccgctgg
ttgcgggcaccccggaccccggtttctggtgcagatgaagtggttgcgtttgccaaagaacatggcctgccgattgcgatcaaagcagcattcggcgg
tggcggtcgcggtctgaaagtggcccgtaccctggaagaagttccggaacttgtatgatagcgcagttcgcgaagcggtggcagcgtttggccgtggt
gaatgcttcgtggaacgctacctggataaaccgcgtcatgttgaaacccagtgtctggcggatacgcaccgcaacgtgcttgtggttagcacccgcg
attgctctctgcaacgtcgccaccagaaactggtggaagaagcaccggcgccgtttctgagcgaagcccagaccgaacagctgtatagctctagtaa
agcgattctgaaagaagccggttacgtggggcgccggtacggttgaatttctggtgggcatggatggcaccattagctttctggaagttaacacccgt
ctgcaagttgaacatccggtgaccgaagaaagttgcgggcattgatctggtgcgcgaaatgtttcgtatccgcagatggccaagaactgggttacgatg
atccggcgctgcgcggtcacagctttgaattctgtattaatggcgaagatccgggccgtggttttctgccggcgccggcgcaccgtgacgctgttcga
tgcaccgaccggtccgggcgttcgtctggatgccggtgtggaaagtggtagcgttattggcccggcatggatagcctgctggcgaaactgatcgtt
accggtcgtacgcgcgccgaagcgctgcaacgtgcagcacgtgccctggatgaatttaccgtggaaggcatggcgacgcccattccgttccatcgca
ccgtggttcgtgatccggcattcgcgccgcgaactgaccggctctaccgatccgttcaccgtgcacacgcgcctggatcgaaaccgaatttgttaacga
aatcaaaccgttcaccacgccggcggataccgaaacggatgaagaaagtggtcgcgaaacggtggttgtcgaagtgggcggtaaacgtctggaagtt
tctctgccgagcagcctgggtatgagtctggcgcgtaccggtctggcggccggcgcccgtccgaaacgtcgcgcagcgaaaaatctggtccggccg
cagcggtgatacccctggccagtccgatgcagggcacgattgtgaaaatcgcagtggaagaaggtcaggaagtgcaggaaggcgatctgattgttgt
gctggaagcgatgaaaatggaacagccgctgaatgcccatcgtagcggcaccatcaaaggcctgacggccgaagtgggtgcatctctgaccagtggc
gcggccatttgcgaaatcaaagattaa
```

Figure 13: Element3: Spacer sequence (Restriction sites and phage T7 gene10 ribosome binding site) (SEQ.ID NO. 13)

```
agatctgcggccgcatctagaaataattttgtttaactttaagaaggagatatattc
```

Figure 14: Element4: Optimized *pccB* (SEQ.ID NO: 14)

```
atgagtgaaccggaagaacagcagccggatattcataccacgcgcaggcaaactggcggatctgcgtcgccgtatcgaacaagcaacccatgcaggta
gcgcacgtgcagtggaaaaacagcacgcgaaaggtaaactgacggcccgcgaacgtatcgatctgctgctggatgaagccagttttgttgaactgga
tgaatttgcacgccaccgtagcaccaacttcggtctggatgcgaatcgcccgtatggcgatggtgtggttaccggttacggtacggtggatggtcgt
ccggtggcagttcttagccaggattttaccgtgttcggcggtgcactgggcgaagtttacggtcagaaaatcgtgaaagctatggatttcgcgctga
aaacgggctgcccggtggttggtattaacgatagcggcggtgcccgcatccaggaaggtgttgcctctctgggcgcgtatggcgaaatctttcgccg
taatacccatgcgagtggcgtgattccgcagatcagcctggtggttggtccgtgtgcgggcggtgccgtttactctccggccattaccgattttacg
gtgatggttgatcagaccagtcacatgttcattacgggcccggatgtgatcaaaaccgttacgggcgaagatgtgggtttgaagaacggcggtg
cacgtacccacaacagcacgtctggcgttcgcatcacatggccggtgatgaaaaagatgccgtggaatatgttaaacagctgctgagttacctgcc
gagcaacaatctgtctgaaccgccggcgttcccggaagaagcagacctggcggtgaccgatgaagatgccgaactggatacgatcgttccggattct
gcaaatcagccgtacgatatgcacagtgtgattgaacacgttctggatgatgcggaatttttcgaaacccagccgctgtttgccccgaacattctga
cgggtttcggtcgtgtggaaggtcgtccggcggtatcgttgcaaatcagccgatgcagtttgcgggttgcctggtatattaccgcctccgaaaaagc
ggcccgctttgtgcgtacctgtgatgcgttcaacgtgccggttctgacgtttgtggatgttccgggcttcctgccgggtgttgatcaggaacatgat
ggcattatccgccgtggtgcgaaactgatttttgcgtatgccgaagcaaccgtgccgctgattaccgttatcacgcgcaaagcattcggcggtgcgt
acgatgtgatgggcagcaaacatctgggtgccgatctgaacctggcatggccgaccgcacagatcgcagtgatgggcgcgcaggtgccgttaatat
tctgcaccgccgtaccatcgcagatgcaggtgatgatgcagaagcgacgcgcgcacgtctgattcaggaatatgaagatgcgctgctgaacccgtat
accgcagcggaacgtggttacgtggatgccgttattatgccgagcgataccgccgtcatatcgtgcgtggtctgcgtcagctgcgtacgaaacgtg
aatctctgccgccgaaaaaacacggtaatattccgctgtaa
```

Figure 15: Entire synthetic sequence for propionyl-CoA carboxylase gene expression. (SEQ.ID NO: 15)

```
aattgtgagcggataacaattgacattgtgagcggataacaagatactgagcacatcagcaggacgcactgaccgaattcaataatttngtttaact
ttaagaaggagatatacatatgcgcaaagtgctgattgcgaaccgtggtgaaatcgccgttcgtgtggcacgcgcgtgtcgtgatgcaggtattgca
agtgttgcggtgtatgccgatccggatcgcgatgcgctgcatgttcgtgcggccgatgaagcctttgcactgggcggtgataccccggcaacgagct
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ggcagcgtttggccgtggtgaatgcttcgtggaacgctacctggataaaccgcgtcatgttgaaaccccagtgtctggccgatacgcacggcaacgtg
gttgtggttagcacccgcgattgctctctgcaacgtcgccaccagaaactggtggaagaagcaccggcgccgtttctgagcgaagcccagaccgaac
agctgtatagctctagtaaagcgattctgaaagaagccggttacgtgggcgccggtacggttgaatttctggtgggcatggatggcaccattagctt
tctggaagttaacacccgtctgcaagttgaacatccggtgaccgaagaagttgcgggcattgatctggtgcgcgaaatgnttcgtatcgcagatggc
gaagaactgggttacgatgatccggcgctgcgcggtcacagctttgaatttcgtattaatggcgaagatccgggccgtggttttctgccggcgccgg
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aaaccgaatttgttaacgaaatcaaaccgtncaccacgccggcggataccgaaacggatgsagaaagtggtcgcgaaacggtggttgtggaagtggg
cggtaaacgtctggaagttttctctgccgagcagcctgggnatgagtctggcgcgtaccggtctggcggccggcgcccgtccgaaacgtcgccgcagcg
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aaggcgatctgattgttgtgctggaagcgangaaaatggaacagccgctgaatgcccatcgtagcggcaccatcaaaggcctgacggccgaagtggg
tgcatctctgaccagtggcgcggccatttgcgaaatcaaagattaaagatctgcggccgcatctagaaataattttgtttaactttaagaaggagat
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tttacggtgatggttgatcagaccagtcacatgttcattacggcccggatgtgatcaaaancgttacggcgcgaagatgtgggttttgaagaactgg
gcggtgcacgtacccacaacagcacgtctcgcgttgcgcatcacatggccggtgatgaaaaagatgccgtggaatatgttaaacagctgctgaqtta
cctgccgagcaacaatctgtctgaaccgccggcgttcccggaagaagcagacctggcggtgaccgatgaagatgccgaactggatacgatcgttccg
gattctgcaaatcagccgtacgatatgcacagtgtgattgaacacgttctggatgatgcggaatttttcgaaacccagccgctgtttgccccgaaca
ttctgacgggtttcggtcgtgtggaaggtcgtccggtgggtatcgttgcaaatcagccgatgcagtttgcgggttgcctggatattaccgcctctga
aaaagcggcccgctttgtgcgtacctgtgangcgttcaacgtgccggttctgacgtttgtggatgttccgggcttcctgccgggtgttgatcaggaa
catgatggcattatccgccgtggtgcgaaactgatttttgcgtatgccgaagcaaccgtgccgctgattaccgttatcacgcgcaaagcattcggcg
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aacgtgaatctctgccgccgaaaaaacaccgtaatattccgctgtaa
```

Figure 16: Forward primer for PrpE (SEQ.ID NO: 16)

AAACTGCAGAGGAGGACAGCTATGTCTTTTAGCGAATTTTATCAG

Figure 17: Reverse primer for PrpE (SEQ.ID NO 17)

AAAGGATCCCTATTCTTCGATCGCCTGGCGAATTTG

Figure 18: MMAT domain sequence from *Mycobacterium bovis* BCG (SEQ.ID NO: 18)

```
LVEGLREVADGDALYDAAVGHGDRGPVWVFSGQGSQWAAMGTQLLASEPVFAATIAKLEP
VIAAESGFSVTEAITAQQTVTGIDKVQPAVFAVQVALAATMEQTYGVRPGAVVGHSMGES
AAAVVAGALSLEDAARVICRRSKLMTRIAGAGAMGSVELPAKQVNSELMARGIDDVVVSV
VASPQSTVIGGTSDTVRDLIARWEQRDVMAREVAVDVASHSPQVDPILDDLAAALADIAP
MTPKVPYYSATLFDPREQPVCDGAYWVDNLRNTVQFAAAVQAAMEDGYRVFAELSPHPLL
THAVEQTGRSLDKSVAALAGMRREQPLPHGLRGLLTELHRAGAALDYSALYPAGRLVDAP
LPAWTHARLFIDDDGQEQRAQGA
```

Figure 19: Protein sequence for the *Mycobacterium bovis* BCG MAS (YP_979046) (SEQ.ID NO: 19)

MESRVTPVAVIGMGCRLPGGINSPDKLWESLLRGDDLVTEIPPDRWDADDYYDPEPGVPGRSVSRWGGFL
DDVAGFDAEFFGISEREATSIDPQQRLLLETSWEAIEHAGLDPASLAGSSTAVFTGLTHEDYLVLTTTAG
GLASPYVVTGLNNSVASGRIAHTLGLHGPAMTFDTACSSGLMAVHLACRSLHDGEADLALAGGCAVLLEP
HACVAASAQGMLSSTGRCHSFDADADGFVRSEGCAMVLLKRLPDALRDGNRIFAVVRGTATNQDGRTETL
TMPSEDAQVAVYRAALAAAGVQPETVGVVEAHGTGTPIGDPIEYRSLARVYGAGTPCALGSAKSNMGHST
ASAGTVGLIKAILSLRHGVVPPLLHFNRLPDELSDVETGLFVPQAVTPWPNGNDHTPKRVAVSSFGMSGT
NVHAIVEEAPAEASAPESSPGDAEVGPRLFMLSSTSSDALRQTARQLATWVEEHQDCVAASDLAYTLARG
RAHRPVRTAVVAANLPELVEGLREVADGDALYDAAVGHGDRGPVWVFSGQGSQWAAMGTQLLASEPVFAA
TIAKLEPVIAAESGFSVIEAITAQQTVTGIDKVQPAVFAVQVALAATMEQTYGVRPGAVVGHSMGESAAA
VVAGALSLEDAARVICRRSKLMTRIAGAGAMGSVELPAKQVNSELMARGIDDVVVSVVASPQSTVIGGTS
DTVRDLIARWEQRDVMAREVAVDVASHSPQVDPILDDLAAALADIAPMTPKVPYYSATLFDPREQPVCDG
AYWVDNLRNTVQFAAAVQAAMEDGYRVFAELSPHPLLTHAVEQTGRSLDMSVAALAGMRREQPLPHGLRG
LLTELHRAGAALDYSALYPAGRLVDAPLPAWTHARLFIDDDGQEQRAQGACTLTVHPLLGSHVRLTEEPE
RHVWQGDVGTSVLSWLSDHQVHNVAALPGAAYCEMALAAAAEVFGEAAEVRDLTFEQMLLLDEQTPIDAV
ASIDAPGVVNFTVETNRDGETTRHATAALRAAEDDCPPPGYDITALLQAHPHAVNGTAMRESFAERGVTL
GAAFGGLTTAHTAEAGAATVLAEVALPASIRFQQGAYRIHPALLDACFQSVGAGVQAGTATGGLLLPLGV
RSLRAYGPTRNARYCYTRLTKAFNDGTRGGEADLDVLDEHGTVLLAVRGLRMGTGTSERDERDRLVSERL
LTLGWQQRALPEVGDGEAGSWLLIDTSNAVDTPDMLASTLTDALKSHGPQGTECASLSWSVQDTPPNDQA
GLEKLGSQLRGRDGVVIVYGPRVGDPDEHSLLAGREQVRHLVRITREIAEFEGELPRLFVVTRQAQIVKP
HDSGERANLEQAGLRGLLRVISSEHPMLRTTLIDVDEHTDVERVAQQILSGSEEDETAWRNGDWYVARLT
PSPLGHEERRTAVLDPDHDGMRVQVRRPGDLQTLEFVASDRVPPGPGQIEVAVSMSSINFADVLIAFGRF
PIIDDREPQLGMDFVGVVTAVGEGVTGHQVGDRVGGFSEGGCWRTFLTCDANLAVTLPPGLTDEQAITAA
TAHATAWYGLNDLAQIKAGDKVLIHSATGGVGQAAISIARAKGAEIFATAGNPAKRAMLRDMGVEHVYDS
RSVEFAEQIRRDTDGYGVDIVLNSLTGAAQRAGLELLAFGGRFVEIGKADVYGNTRLGLFPFRRGLTFYY
LDLALMSVTQPDRVRELLATVFKLTADGVLTAPQCTHYPLAEAADAIRAMSNAEHTGKLVLDVPRSGRRS
VAVIPEQAPLYRRDGSYIITGGLGGLGLFFASKLAAAGCGRIVLTARSQPNPKARQTIEGLRAAGADIVV
ECGNIAEPDTADRLVSAATATGLPLRGVLHSAAVVEDATLTNITDELIDRDWSPKVFGSWNLHRATLGQP
LDWFCLFSSGAALLGSPGQGAYAAANSWVDVFAHWRRAQGLPVSAIAWGAWGEVGRATFLAEGGEIMITP
EEGAYAFETLVRHDRAYSGYIPILGAPWLADLVRRSPWGEMFASTGQRSRGPSKFRMELLSLPQDEWAGR
LRRLLVEQASVILRRTIDADRSFIEYGLDSLGMLEMRTHVETETGIRLTPKVLATNNTARALAQYLADTL
AEEQAAAPAAS

Figure 20: Codon-optimized MMAT domain DNA sequence from *Mycobacterium bovis* BCG (SEQ.ID NO: 20)

CTGGTGGAAGGCCTGCGTGAAGTTGCCGATGGTGATGCACTGTATGATGCAGCAGTGGGTCATGGCGAT
CGTGGTCCGGTTTGGGTGTTTAGCGGCCAGGGTTCTCAGTGGGCAGCGATGGGCACCCAGCTGCTGGCAAGCGAA
CCGGTTTTTGCCGCAACGATTGCAAAACTGGAACCGGTGATCGCGGCCGAAAGTGGCTTCAGCGTTACCGAAGCA
ATTACGGCGCAGCAGACCGTGACGGGTATCGATAAAGTGCAGCCGGCCGTTTTCGCAGTTCAGGTGGCGCTGGCA
GCGACGATGGAACAGACGTACGGCGTTCGTCCGGGTGCAGTGGTTGGTCACAGTATGGGTGAAAGCGCCGCAGCG
GTGGTTGCAGGCGCCCTGAGTCTGGAAGATGCCGCACGTGTGATTTGCCGTCGCAGCAAACTGATGACCCGTATC
GCAGGTGCAGGTGCGATGGGCAGCGTGGAACTGCCGGCAAAACAGGTTAACCTGAACTGATGGCGCGCGGTATT
GATGATGTGGTTGTGTCTGTTGTGGCGTCTCCGCAGAGTACCGTGATTGGCGGCACCAGTGATACGGTTCGTGAT
CTGATCGCGCGTTGGGAACAGCGCGATGTGATGGCGCGCGAAGTTGCCGTGGATGTTGCAAGCCATTCTCCGCAG
GTTGATCCGATTCTGGATGATCTGGCGGCGGCACTGGCAGATATTGCACCGATGACCCCGAAAGTGCCGTATTAC
AGCGCGACGCTGTTTGATCCGCGTGAACAGCCGGTGTGTGATGGCGCCTATTGGGTTGATAACCTGCGCAATACC
GTGCAGTTTGCGGCGGCAGTTCAGGCGGCGATGGAAGATGGTTACCGTGTGTTCGCGGAACTGTCTCCGCATCCG
CTGCTGACCCACGCAGTGGAACAGACGGGTCGCTCTCTGGATATGAGTGTTGCAGCACTGGCCGGTATGCGTCGC
GAACAGCCGCTGCCGCATGGCCTGCGTGGTCTGCTGACCGAACTGCACCGTGCAGGTGCAGCACTGGATTATAGC
GCACTGTACCCGGCAGGTCGTCTGGTGGATGCACCGCTGCCGGCATGGACGCACGCACGTCTGTTCATCGATGAT
GATGGCCAGGAACAGCGCGCACAGGGTGCG

Figure 21: Alignment of a codon-optimized MMAT domain from *Mycobacterium bovis* BCG with the original sequence:

```
Optimized    1 CTGGTGGAAGGCCTGCGTGAAGTTGCCGATGGTGATGCACTGTATGATGCAGCAGTGGGT
               || || || ||  |||| || || ||||| ||||| || || ||||| || || |||||
Original     1 CTCGTCGAGGGTTTGCGCGAGGTGGCCGACGGTGACGCCCTCTATGACGCGGCGGTGGGA

Optimized   61 CATGCCGATCGTGGTCCGGTTTGGGTGTTTAGCGGCCAGGGTTCTCAGTGGCAGCGATG
               || || ||||| || || || ||||| ||   ||| || || || |||||||| ||||||
Original    61 CACGGTGATCGAGGACCGGTCTGGGTCTTCTCCGGGCAAGGGTCGCAGTGGCCGGCGATG

Optimized  121 GGCACCCAGCTGCTGGCAAGCGAACCGGTTTTTGCCGCAACGATTGCAAAACTGGAACCG
               ||||| ||  |||| || |||||||| || || || || || || || || ||||| |||
Original   121 GGCACGCAATTGCTCGCCAGCGAACCAGTGTTCGCGGCCACCATCGCCAAGCTGGAGCCG

Optimized  181 GTGATCGCGGCCGAAAGTGGCTTCAGCGTTACCGAAGCAATTACGGCGCAGCAGACCGTG
               |||||||| || |||   || |||   || ||||| || || ||||||||||||||||||
Original   181 GTGATCGCCGCAGAATCGGGATTCTCGGTGACCGAGGCGATAACGGCGCAGCAGACCGTG

Optimized  241 ACGGGTATCGATAAAGTGCAGCCGGCCGTTTTCGCAGTTCAGGTGGCGCTGGCAGCGACG
               || || ||||| ||||| |||||||| || ||||| |||||||| ||| |||| || ||
Original   241 ACCGGAATCGACAAAGTGCAGCCGGCAGTGTTCGCCGTTCAGGTCGCGTTGGCCGCCACC

Optimized  301 ATGGAACAGACGTACGGCGTTCGTCCGGGTGCAGTGGTTGGTCACAGTATGGGTGAAAGC
               ||||| || ||  |||| || || ||||| || || || || |||   ||||||||
Original   301 ATGGAGCAAACCTACGGAGTGCGGCCGGGCGCGGTCGTCGGACACTCGATGGGTGAGTCG

Optimized  361 GCCGCAGCGGTGGTTGCAGGCGCCCTGAGTCTGGAAGATGCCGCACGTGTGATTTGCCGT
               ||||| || || || || || || |||   || || || || || || || ||||||||
Original   361 GCCGCGGCCGTCGTCGCGGGGGCACTGTCGCTCGAGGACGCGGCGCGCGTCATTTGCCGC

Optimized  421 CGCAGCAAACTGATGACCCGTATCGCAGGTGCAGGTGCGATGGGCAGCGTGGAACTGCCG
               |||   || |||||||| || || || |||| |||| ||||||   |||||| ||||
Original   421 CGCTCGAAGCTGATGACCCGCATAGCCGGTGCTGGTGCCATGGGCTCGGTGGAATTGCCC

Optimized  481 GCAAAACAGGTTAACTCTGAACTGATGGCGCGCGGTATTGATGATGTGGTTGTGTCTGTT
               || || || || || || || ||||||| ||||| || || ||||| || || || ||
Original   481 GCCAAGCAAGTGAATTCGGAGCTGATGGCACGCGGAATCGACGATGTTGTCGTCTCGGTG

Optimized  541 GTGGCGTCTCCGCAGAGTACCGTGATTGGCGGCACCAGTGATACGGTTCGTGATCTGATC
               |||||||| |||||    || ||||| |||| || || || || |||||||| || |||
Original   541 GTGGCGTCCCCGCAATCCACGGTGATCGGCGGTACGAGCGACACCGTTCGTGACCTCATC

Optimized  601 GCGCGTTGGGAACAGCGCGATGTGATGGCGCGCGAAGTTGCCGTGGATGTTGCAAGCCAT
               || |||||||| |||| || ||||||||||||| || |||| || || ||    ||
Original   601 GCCCGTTGGGAGCAGCGGGACGTGATGGCGCGCGAGGTGGCCGTCGACGTGGCGTCGCAC

Optimized  661 TCTCCGCAGGTTGATCCGATTCTGGATGATCTGGCGGCGGCACTGGCAGATATTGCACCG
               || || || || |||| || || || ||| |||| |||| |||| || |||| |||
Original   661 TCGCCTCAAGTCGATCCGATACTCGACGATTTGGCCGCGGCGCTGGCGGACATTGCTCCG

Optimized  721 ATGACCCCGAAAGTCCCGTATTACAGCCGCGACCCTGTTTGATCCGCGTGAACAGCCGGTG
               ||||| || || |||| || |||   |||| |||| || |||| || |||||||||
Original   721 ATGACGCCCAAGGTGCCGTACTACTCGGCGACCCTGTTCGACCCGCGCGGAGCAGCCGGTG

Optimized  781 TGTGATGGCGCCTATTGGGTTGATAACCTGCGCAATACCGTGCAGTTTGCGGCGGCAGTT
               || |||||||| || || || || || ||||||| || |||||||| || ||||| ||
Original   781 TGCGATGGCGCTTACTGGGTGGACAATCTGCGCAACACGGTGCAGTTCGCCGCGGCGGTG

Optimized  841 CAGGCGGCGATGGAAGATGGTTACCGTGTGTTCGCGGAACTGTCTCCGCATCCGCTGCTG
               ||||| |||||||| || || ||||| || |||||||| ||||| || || ||||||||
Original   841 CAGGCTGCGATGGAGGACGGCTACCGGGTCTTCGCGGAGCTGTCGCCCCACCCGCTGCTT

Optimized  901 ACCCACGCACTGGAACAGACGGGTCGCTCTCTCGATATGAGTGTTGCAGCACTGGCCGGT
               |||||||| || ||||| ||||| ||    || || |||   || || || ||||||||
Original   901 ACCCACGCCGTCGAACAGACGGGCCGAAGCCTCGACATGTCGGTCGCCGCCCTGGCCGGC
```

```
Optimized  961 ATGCGTCGCGAACAGCCGCTGCCGCATGGCCTGCGTGGTCTGCTGACCGAACTGCACCGT
               ||||| || || ||||| |||||||||||| ||||| ||  ||||||| || ||||||||
Original   961 ATGCGGCGAGAGCAGCCTCTGCCGCATGGTCTGCGCGGCTTGCTGACGGAGCTGCACCGC

Optimized 1021 GCAGGTGCAGCACTGGATTATAGCGCACTGTACCCGGCAGGTCGTCTGGTGGATGCACCG
               || || || ||  |||| |||   || ||||| || || || || |||||||||||| |||
Original  1021 GCGGGCGCCGCTTTGGACTATTCGGCGCTGTATCCCGCTGGGCGGCTGGTGGATGCGCCG

Optimized 1081 CTGCCGGCATGGACGCACGCACGTCTGTTCATCGATGATGATGGCCAGGAACAGCGCGCA
               |||||||| ||||| ||||| || || |||||||| |||||||| || |||||||| |||
Original  1081 CTGCCGGCGTGGACCCACGCCCGCCTATTCATCGACGATGATGGGCAAGAACAGCGGGCA

Optimized 1141 CAGGGTGCG
               || |||||
Original  1141 CAAGGTGCC
```

Figure 22: Protein sequence of *Salmonella enterica* propionyl CoA synthase PrpE (AAC44817) (SEQ.ID NO. 21)

MSFSEFYQRSINEPEAFWAEQARRIDWRQPFTQTLDHSRPPFARWFCGGTTNLCHNAVDRWRDKQPEALA
LIAVSSETDEERTFTFSQLHDEVNIVAAMLLSLGVQRGDRVLVYMPMIAEAQITLLACARIGAIHSVVFG
GFASHSVAARIDDARPALIVSADAGARGGKILPYKKLLDDAIAQAQHQPKHVLLVDRGLAKMAWVDGRDL
DFATLRQQHLGASVPVAWLESNETSCILYTSGTTGKPKGVQRDVGGYAVALATSMDTIFGGKAGGVFFCA
SDIGWVVGHSYIVYAPLLAGMATIVYEGLPTYPDCGVWWKIVEKYQVNRMFSAPTAIRVLKKFPTAQIRN
HDLSSLEALYLAGEPLDEPTASWVTETLGVPVIDNYWQTESGWPIMALARALDDRPSRLGSPGVPMYGYN
VQLLNEVTGEPCGINEKGMLVIEGPLPPGCIQTIWGDDARFVKTYWSLFNRQVYATFDWGIRDAEGYYFI
LGRTDDVINIAGHRLGTREIEESISSYPNVAEVAVVGIKDALKGQVAVAFVIPKQSDTLADREAARDEEN
AIMALVDNQIGHFGRPAHVWFVSQLPKTRSGKMLRRTIQAICEGRDPGDLTTIDDPASLQQIRQAIEE

Figure 23: DNA sequence of *Salmonella enterica* propionyl CoA synthase PrpE (SEQ.ID NO. 22)

ATGTCTTTTAGCGAATTTTATCAGCGTTCCATTAACGAACCGGAGGCGTTCTGGGCCGAG
CAGGCCCGGCGTATCGACTGGCGACAGCCGTTTACGCAGACGCTGGATCATAGCCGTCCA
CCGTTTGCCCGCTGGTTTTGCGGCGGCACCACTAACTTATGTCATAACGCCGTCGACCGC
TGGCGGGATAAACAGCCGGAGGCGCTGGCGCTGATTGCCGTCTCATCAGAGACCGATGAA
GAGCGCACATTTACCTTCAGCCAGTTGCATGATGAAGTCAACATTGTGGCCGCCATGTTG
CTGTCGCTGCGCGTGCAGCGTCGCGATCGCGTATTGGTCTATATGCCGATGATTGCCGAA
GCGCAGATAACCCTGCTGGCCTGCGCGCGCATTGGCGCGATCCATTCGGTGGTCTTTGGC
GGTTTTGCCTCGCACAGCGTGGCGGCGCGCATTGACGATGCCAGACCGGCGCTGATTGTG
TCGGCGGATGCCGGAGCGCGGGGCGGTAAAATCCTGCCGTATAAAAAGCTGCTCGATGAC
GCTATTGCGCAGGCGCAGCATCAGCCGAAACACGTTCTGCTGGTGGACAGAGGGCTGGCG
AAAATGGCATGGGTGGATGGGCGCGATCTGGATTTTGCCACGTTGCGCCAGCAGCATCTC
GGCGCGAGCGTGCCGGTGGCGTGGCTGGAATCCAACGAAACCTCGTGCATTCTTTACACC
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CTGGCAACCTCGATGGACACCATTTTTGGCGGCAAGGCGGGCGGCGTATTCTTTTGCGCA
TCGGATATCGGCTGGGTCGTCGGCCACTCCTATATCGTTTACGCGCCGTTGCTGGCAGGC
ATGGCGACTATTGTTTACGAAGGACTGCCGACGTACCCGGACTGCGGGGTCTGGTGGAAA
ATTGTCGAGAAATACCAGGTTAACCGGATGTTTTCCGCCCCGACCGCGATTCGCGTGCTG
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CTGGCCGGTCAGCCGCTGGACCAGCCGACGGCCAGTTGGGTAACGGAGACGCTGGGCGTA
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GCGCTGGACGACAGGCCGTCGCGTCTGGGAAGTCCCGGCGTGCCGATGTACGGTTATAAC
GTCCAGCTACTCAATGAAGTCACCGGCGAACCTTGCGGCATAAATGAAAAGGGGATGCTG
GTGATCGAAGGGCCGCTGCCGCCGGGCTGTATTCAGACTATTTGGGGCGACGATGCGCGT
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ATCCGCGACGCCGAGGGGTATTACTTTATTCTGGGCCGTACCGATGATGTGATTAATATT
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GCGGAAGTGGCGGTAGTGGGGATAAAAGACGCTCTGAAAGGGCAGGTAGCGGTGGCGTTT
GTCATTCCGAAGCAGAGCGATACGCTGGCGGATCGCGAGGCGGCGCGCGACGAGGAAAAC
GCGATTATGGCGCTGGTGGACAACCAGATCGGTCACTTTGGTCGTCCGGCGCATGTCTGG
TTTGTTTCGCAGCTCCCCAAAACGCGTTCCGGAAAGATGCTTCGCCGCACGATCCAGGCG
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CAAATTCGCCAGGCGATCGAACAA

Figure 24

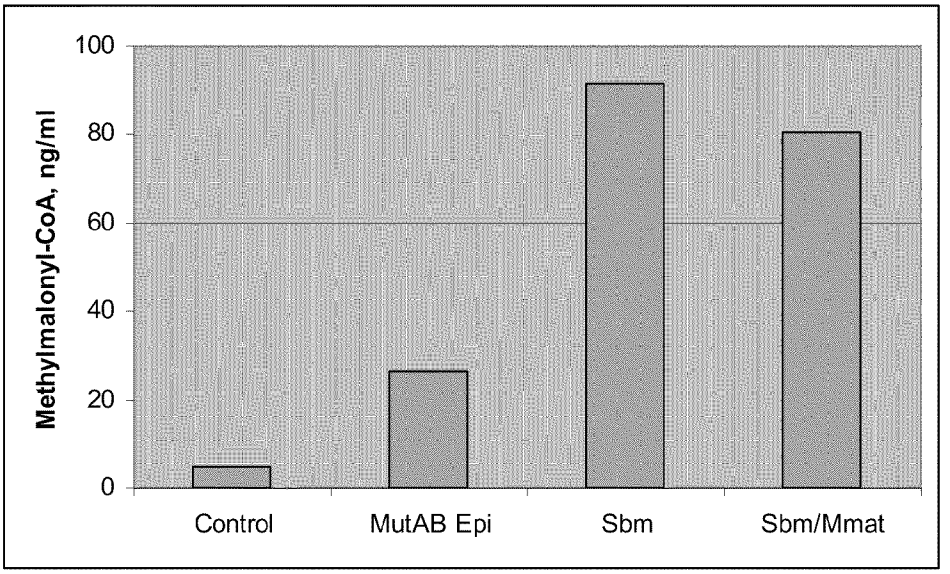


Figure 25

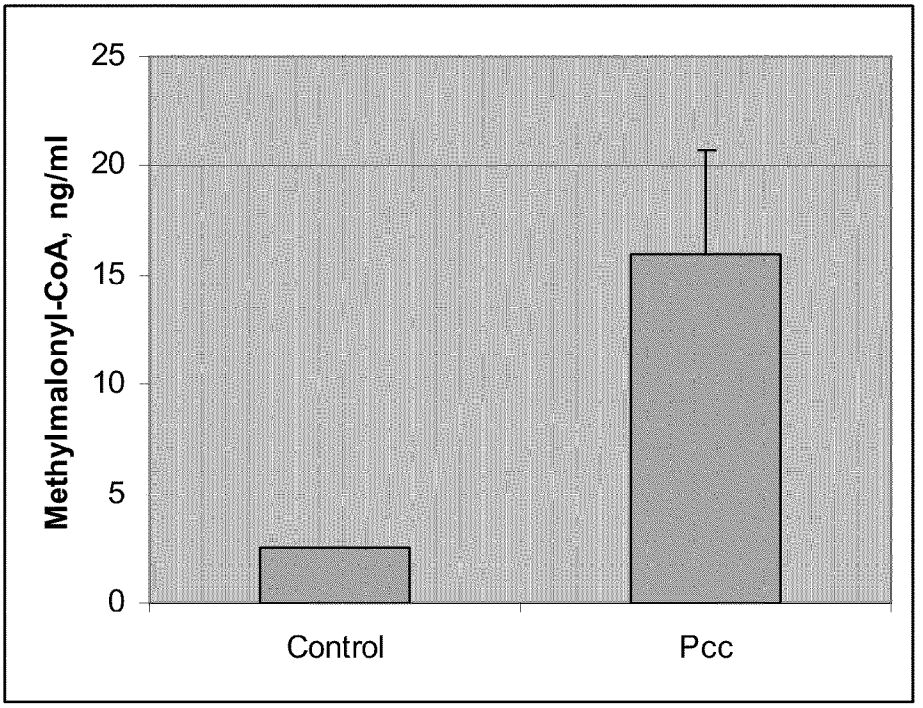


Figure 26

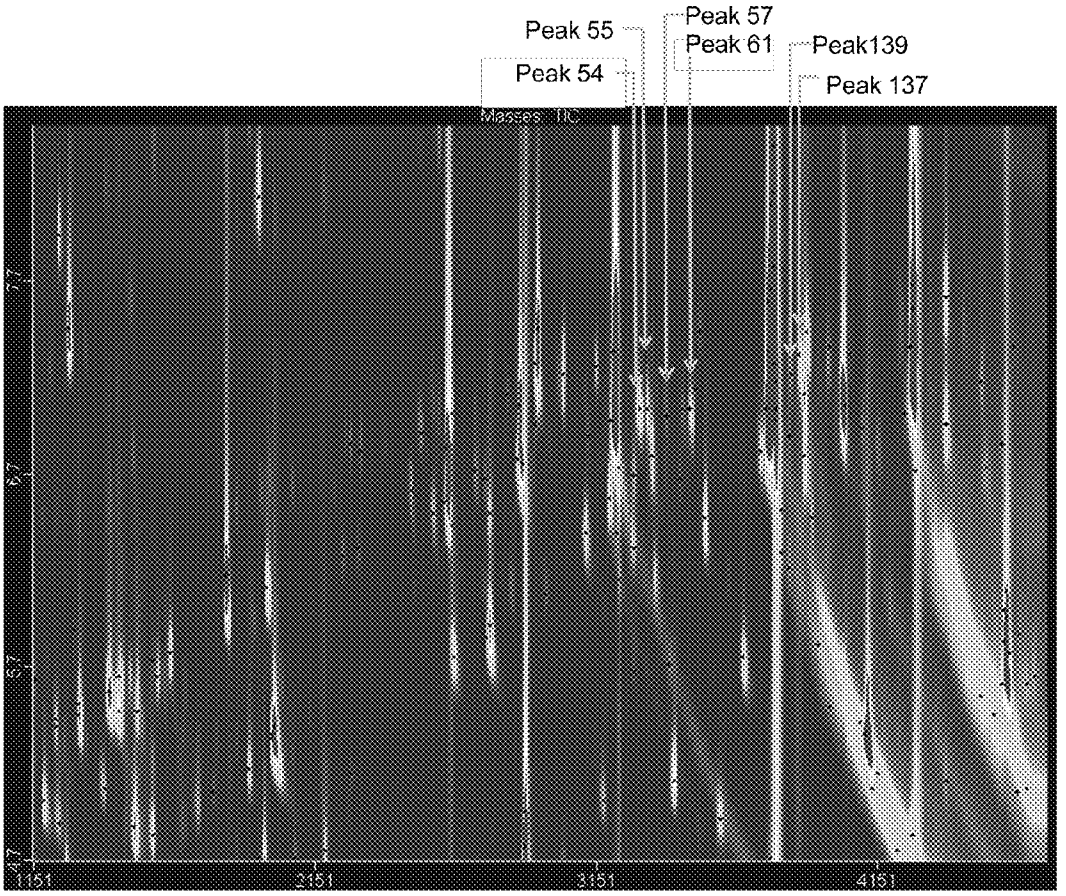

Peak 55
Peak 57
Peak 61
Peak139
Peak 54
Peak 137
Masses TIC
1151
2151
3151
4151

Figure 27

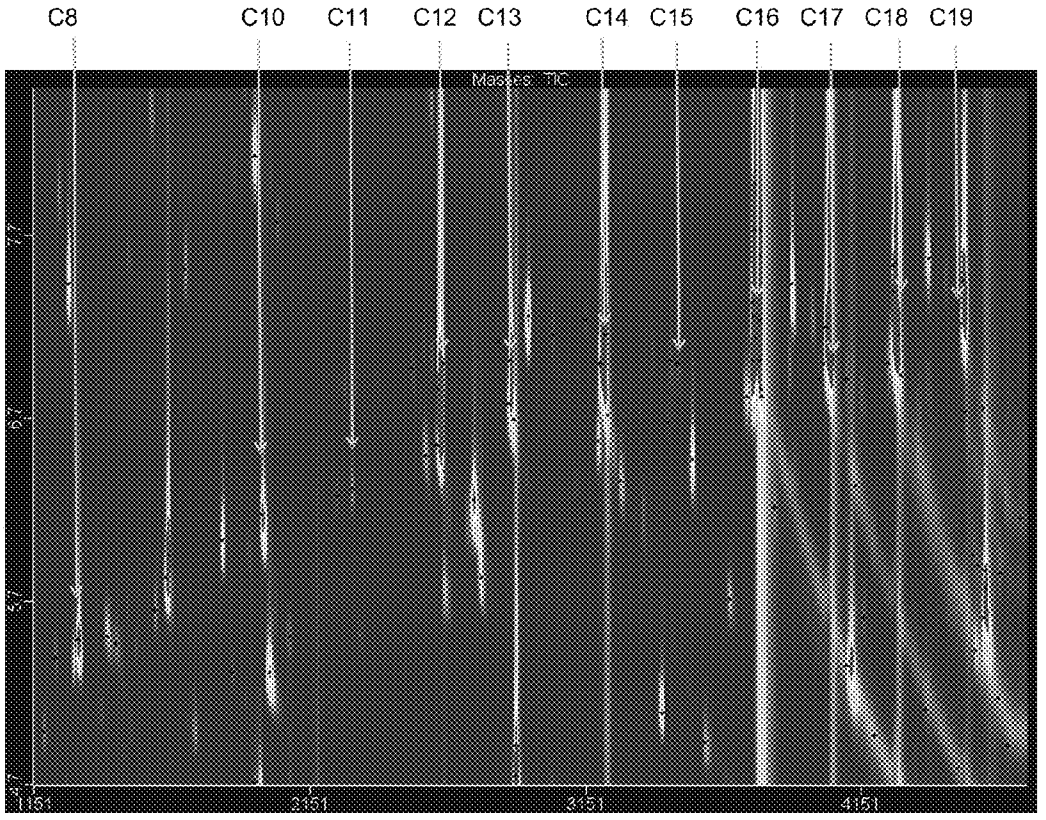
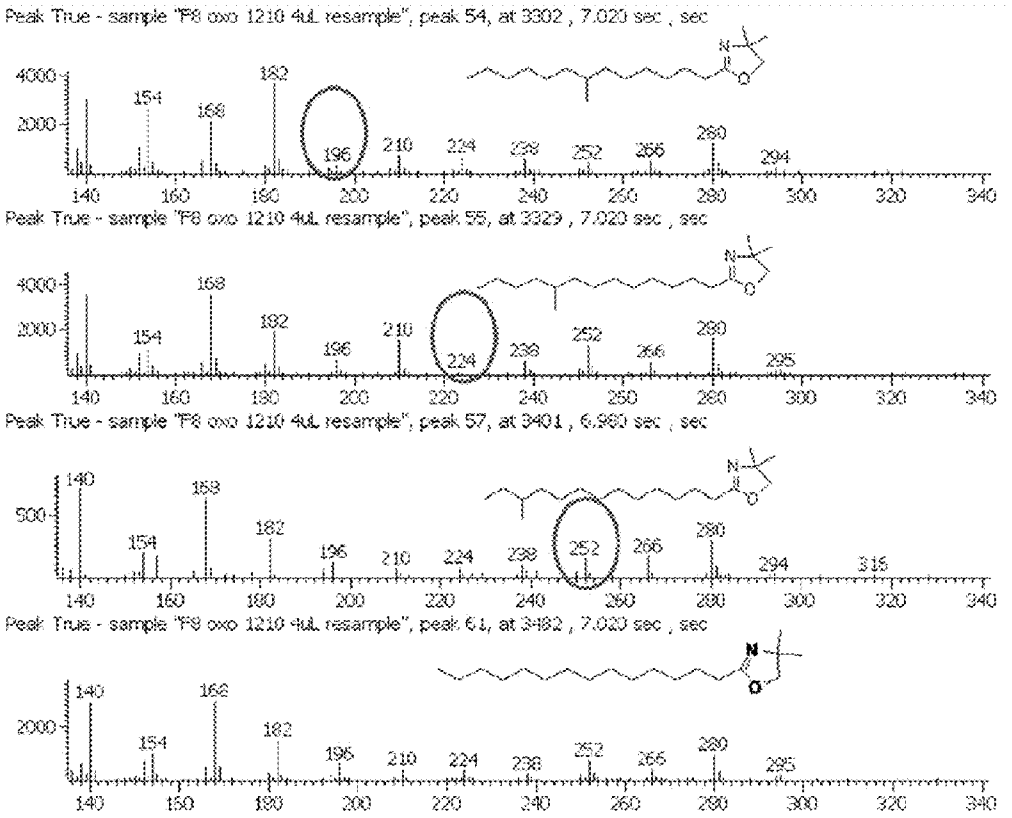

C8
C10
C11
C12
C13
C14
C15
C16
C17
C18
C19
Masses TIC
1151
2151
3151
4151

Figure 28


Peak True - sample "F8 oxo 1210 4uL resample", peak 54, at 3302 , 7.020 sec , sec
Peak True - sample "F8 oxo 1210 4uL resample", peak 55, at 3329 , 7.020 sec , sec
Peak True - sample "F8 oxo 1210 4uL resample", peak 57, at 3401 , 6.980 sec , sec
Peak True - sample "F8 oxo 1210 4uL resample", peak 61, at 3482 , 7.020 sec , sec

SCATTERED BRANCHED-CHAIN FATTY ACIDS AND BIOLOGICAL PRODUCTION THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS AND INCORPORATION BY REFERENCE

[0001] This application claims priority to U.S. Provisional Patent Application No. 61/294,274, filed Jan. 12, 2010, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates to cells and methods for producing fatty acids, and more particularly relates to cells and methods for producing scattered branched-chain fatty acids.

BACKGROUND OF THE INVENTION

[0003] Branched-chain fatty acids are carboxylic acids with a methyl or ethyl branch on one or more carbons that can be either chemically synthesized or isolated from certain animals and bacteria. While certain bacteria, such as *Escherichia coli*, do not naturally produce branched-chain fatty acids, some bacteria, such as members of the genera *Bacillus* and *Streptomyces*, can naturally produce these fatty acids. For example, *Streptomyces avermitilis* and *Bacillus subtilis* both produce branched-chain fatty acids with from 14 to 17 total carbons, with the branches in the iso and anteiso positions (Cropp et al., *Can. J. Microbiology* 46: 506-14 (2000); De Mendoza et al., Biosynthesis and Function of Membrane Lipids, in *Bacillus subtilis* and Other Gram-Positive Bacteria, Sonenshein and Losick, eds., American Society for Microbiology (1993)). However, these organisms do not produce branched-chain fatty acids in amounts that are commercially useful. Another limitation of these natural organisms is that they apparently do not produce medium-chain branched-chain fatty acids, such as those with 11 or 13 carbons. In addition, if fatty acids having particular chain lengths, branches on particular carbons, or branches at positions other than the iso and anteiso positions are desired, these fatty acids may not be available or easily isolated from a natural organism in meaningful quantities.

[0004] As such, there remains a need for commercially useful, bacterially-produced, branched-chain fatty acids. In addition, there remains a need for a method of producing such branched-chain fatty acids.

SUMMARY OF THE INVENTION

[0005] Methods and cells for producing scattered branched-chain fatty acids are provided. In certain embodiments, the method for producing branched-chain fatty acids in a cell includes expressing in the cell one or more recombinant polypeptides that catalyze the conversion of methylmalonyl-CoA to methylmalonyl-ACP; and culturing the cell under conditions suitable for producing the polypeptide, such that branched-chain fatty acids are produced.

[0006] Also provided is a method for producing branched-chain fatty acids in a cell, the method including expressing in the cell one or more recombinant polypeptides that increase the production of methylmalonyl-CoA in the cell; and culturing the cell under conditions suitable for producing the recombinant polypeptide, such that branched-chain fatty acids are produced.

[0007] In certain embodiments, a method for producing branched-chain fatty acids in a cell is provided, the method including expressing in the cell a polypeptide that has propionyl-CoA synthetase activity; inhibiting propionylation of the propionyl-CoA synthetase; and culturing the cell under conditions suitable for producing the polypeptide, such that branched-chain fatty acids are produced.

[0008] Further provided is a method for producing branched-chain fatty acids in a cell, the method including expressing in the cell a polypeptide that has methylmalonyl-CoA mutase activity; expressing in a cell a polypeptide that has methylmalonyl-CoA epimerase activity; and culturing the cell under conditions suitable for producing the polypeptides, such that branched-chain fatty acids are produced.

[0009] A composition comprising a mixture of biologically-produced branched-chain fatty acids is also provided. The composition can include branched-chain fatty acids having a chain length of C12 to C16 and from about 1 to about 3 methyl branches positioned on one or more even-numbered carbons.

[0010] In certain embodiments, a method for producing branched-chain fatty acids in a cell is provided, the method including expressing in the cell one or more recombinant polypeptides that increase the production of methylmalonyl-CoA in the cell; expressing in the cell a recombinant polypeptide that catalyzes the conversion of methylmalonyl-CoA to methylmalonyl-ACP; and culturing the cell under conditions suitable for producing the recombinant polypeptide, such that branched-chain fatty acids are produced.

[0011] In addition, in certain embodiments, a method for producing branched-chain fatty acids in a cell is provided, the method including expressing in the cell one or more recombinant polypeptides that increase the production of methylmalonyl-CoA in the cell; expressing in the cell a recombinant polypeptide that catalyzes the conversion of methylmalonyl-CoA to methylmalonyl-ACP; expressing in the cell a recombinant thioesterase; and culturing the cell under conditions suitable for producing the recombinant polypeptide, such that branched-chain fatty acids are produced.

[0012] Also provided is a method for producing branched-chain fatty acids in a cell, the branched-chain fatty acids having a chain length from about 10 to 18 carbons and branching at the second carbon. The method includes modifying the cell to increase carbon flow to methylmalonyl-CoA; and culturing the cell under conditions suitable for carbon flow to methylmalonyl-CoA to be increased, such that branched-chain fatty acids having a chain length from about 10 to about 18 carbons and branching at the second carbon are produced. In certain embodiments, the branching can be on the fourth, sixth, eighth, tenth, or twelfth carbon.

[0013] In certain embodiments, a method for producing branched-chain fatty acids in a cell is provided, the branched-chain fatty acids having a chain length from about 10 to 18 carbons and branching at the second carbon. The method includes modifying the cell to generate methylmalonyl-ACP from methylmalonyl-CoA; and culturing the cell under conditions suitable for generation of methylmalonyl-ACP from methylmalonyl-CoA, such that branched-chain fatty acids having a chain length from about 10 to about 18 carbons and branching at the second carbon are produced. In certain embodiments, the branching can be on the fourth, sixth, eighth, tenth, or twelfth carbon.

[0014] A method for producing modified fatty acids in a cell is also provided, the method including providing a cell

having type II fatty acid synthase activity; expressing in the cell one or more recombinant polypeptides that catalyze formation of at least one intermediate metabolite, wherein the at least one intermediate metabolite is incorporated by the type II fatty acid synthase; and culturing the cell under conditions suitable for producing the recombinant polypeptide, such that modified fatty acids are produced.

[0015] Further provided is an *Escherichia* cell that produces branched-chain fatty acids having a chain length from about 10 to about 18 carbons and comprising one or more methyl branches on one or more even-numbered carbons.

[0016] The invention further provides a method for producing branched-chain fatty acid comprising a methyl on one or more even number carbons. The method comprises culturing a cell comprising (aa) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a polypeptide that catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA and/or (bb) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a polypeptide that catalyzes the conversion of succinyl-CoA to methylmalonyl-CoA. The cell is cultured under conditions allowing expression of the polynucleotide(s) and production of branched-chain fatty acid. Optionally, the method further comprises extracting from the culture the branched-chain fatty acid or a product of the branched-chain fatty acid. Also provided is a cell comprising (i) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding an acyl transferase lacking polyketide synthesis activity, and (ii) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a propionyl-CoA carboxylase and/or an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA mutase, which are expressed in the cell. The cell produces more branched-chain fatty acid comprising a methyl on one or more even number carbons than an otherwise similar cell that does not comprise the polynucleotide(s).

[0017] The following numbered paragraphs each succinctly define one or more exemplary variations of the invention:

[0018] 1. A method for producing branched-chain fatty acid comprising a methyl on one or more even number carbons, the method comprising culturing a cell comprising

[0019] (aa) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a polypeptide that catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA and/or (bb) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a polypeptide that catalyzes the conversion of succinyl-CoA to methylmalonyl-CoA, under conditions allowing expression of the polynucleotide(s) and production of branched-chain fatty acid, wherein the cell produces more fatty acid comprising a methyl on one or more even number carbons than an otherwise similar cell that does not comprise the polynucleotide(s).

[0020] 2. The method of paragraph 1 further comprising extracting from culture the branched-chain fatty acid or a product of the branched-chain fatty acid.

[0021] 3. The method of paragraph 1 or paragraph 2, wherein the polypeptide that catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA is a propionyl-CoA carboxylase and/or the polypeptide that catalyzes the conversion of succinyl-CoA to methylmalonyl-CoA is a methylmalonyl-CoA mutase.

[0022] 4. The method of paragraph 3, wherein (i) the propionyl-CoA carboxylase is *Streptomyces coelicolor* PccB and AccA1 or PccB and AccA2 and/or (ii) the methylmalonyl-CoA mutase is *Janibacter* sp. HTCC2649 methylmalonyl-CoA mutase, *S. cinnamonensis* MutA and MutB, or *E. coli* Sbm.

[0023] 5. The method of paragraph 3, wherein (i) the methylmalonyl-CoA mutase comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence set forth in SEQ ID NOs: 3, 4, or 28 and/or (ii) the propionyl-CoA carboxylase comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence set forth in SEQ ID NOs: 9 and 10.

[0024] 6. The method of any one of paragraphs 3-5, wherein the cell comprises an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA mutase and further comprises an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA epimerase.

[0025] 7. The method of any one of paragraphs 1-6, wherein the cell further comprises an exogenous or overexpressed polynucleotide encoding an acyl transferase lacking polyketide synthesis activity and/or an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a thioesterase.

[0026] 8. The method of paragraph 7, wherein the acyl transferase is FabD, an acyl transferase domain of a polyketide synthase, or an acyl transferase domain of *Mycobacterium* mycocerosic acid synthase.

[0027] 9. The method of any one of paragraphs 1-8, wherein the cell has been modified to attenuate endogenous methylmalonyl-CoA mutase activity, endogenous methylmalonyl-CoA decarboxylase activity, and/or endogenous acyl transferase activity.

[0028] 10. The method of any one of paragraphs 1-9, wherein the cell produces a Type II fatty acid synthase.

[0029] 11. The method of any one of paragraphs 1-10, wherein the cell is *Escherichia coli.*

[0030] 12. A branched-chain fatty acid produced by the method of any one of paragraphs 1-11.

[0031] 13. A cell comprising: (i) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding an acyl transferase lacking polyketide synthesis activity, and (ii) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a propionyl-CoA carboxylase and/or an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA mutase, wherein the polynucleotide(s) are expressed and the cell produces more branched-chain fatty acid comprising a methyl on one or more even number carbons than an otherwise similar cell that does not comprise the polynucleotide(s).

[0032] 14. The cell of paragraph 13, wherein (i) the propionyl-CoA carboxylase is *Streptomyces coelicolor* PccB and AccA1 or PccB and AccA2 and/or (ii) the methylmalonyl-CoA mutase is *Janibacter* sp. HTCC2649 methylmalonyl-CoA mutase, *S. cinnamonensis* MutA and MutB, or *E. coli* Sbm.

[0033] 15. The cell of paragraph 13, wherein (i) the methylmalonyl-CoA mutase comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence set forth in SEQ ID NOs: 3, 4, or 28 and/or (ii) the propionyl-CoA carboxylase comprises an amino acid

sequence having at least about 80% sequence identity to the amino acid sequence set forth in SEQ ID NOs: 9 and 10.

[0034] 16. The cell of any one of paragraphs 13-15, wherein the cell comprises an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA mutase and further comprises an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA epimerase.

[0035] 17. The cell of any one of paragraphs 13-16, wherein the acyl transferase is FabD, an acyl transferase domain of a polyketide synthase, or an acyl transferase domain of *Mycobacterium* mycocerosic acid synthase.

[0036] 18. The cell of any one of paragraphs 13-17, wherein the cell further comprises an exogenous or overexpressed polynucleotide comprises a nucleic acid sequence encoding a thioesterase.

[0037] 19. The cell of any one of paragraphs 13-18, wherein the cell has been modified to attenuate endogenous methylmalonyl-CoA mutase activity, endogenous methylmalonyl-CoA decarboxylase activity, and/or endogenous acyl transferase activity.

[0038] 20. The cell of any one of paragraphs 13-19, wherein the cell is *Escherichia coli.*

[0039] 21. A method for producing branched-chain fatty acids in a cell comprising: a. expressing in the cell one or more recombinant polypeptides that catalyze the conversion of methylmalonyl-CoA to methylmalonyl-ACP; and b. culturing the cell under conditions suitable for producing the polypeptide, such that branched-chain fatty acids are produced.

[0040] 22. The method of paragraph 21, wherein the polypeptide is an acyl transferase.

[0041] 23. The method of paragraph 21, wherein the polypeptide is encoded by fabD.

[0042] 24. The method of paragraph 22, wherein the polypeptide is a polyketide synthase or a portion thereof.

[0043] 25. The method of paragraph 21, wherein the polypeptide is a *Mycobacterium* mycocerosic acid synthase or a portion thereof

[0044] 26. The method of paragraph 21, wherein the polypeptide has at least about 60% sequence identity to a sequence set forth in SEQ ID NO: 19.

[0045] 27. The method of paragraph 21, wherein the method further includes expressing in the cell a polypeptide that encodes an exogenous thioesterase.

[0046] 28. The method of paragraph 21, wherein the cell is an *Escherichia* cell.

[0047] 29. The method of paragraph 21, wherein the cell produces higher levels of branched-chain fatty acids after expression of the polypeptide than it did prior to expression of the polypeptide.

[0048] 30. The method of paragraph 21, wherein the branched-chain fatty acids comprise one or more methyl branches.

[0049] 31. The method of paragraph 30, wherein the one or more methyl branches are on even numbered carbons.

[0050] 32. The method of paragraph 21, wherein the branched-chain fatty acids are not naturally produced in the cell.

[0051] 33. Branched-chain fatty acids produced by the method of paragraph 21.

[0052] 34. A cell comprising at least one recombinant polypeptide that catalyzes the conversion of methylmalonyl-CoA to methylmalonyl-ACP, wherein the cell comprising the recombinant polypeptide produces more branched-chain fatty acids than an otherwise similar cell that does not comprise the recombinant polypeptide.

[0053] 35. A method for producing branched-chain fatty acids in a cell comprising: a. expressing in the cell one or more recombinant polypeptides that increase the production of methylmalonyl-CoA in the cell; and b. culturing the cell under conditions suitable for producing the recombinant polypeptide, such that branched-chain fatty acids are produced.

[0054] 36. The method of paragraph 35, wherein expression of the polypeptide results in increased propionyl-CoA synthetase activity in the cell.

[0055] 37. The method of paragraph 35, wherein the polypeptide has propionyl-CoA carboxylase activity.

[0056] 38. The method of paragraph 35, wherein the polypeptide has at least about 60% sequence identity to a sequence set forth in SEQ ID NO: 9 or SEQ ID NO: 10.

[0057] 39. The method of paragraph 35, wherein the method further includes expressing in the cell a polypeptide that encodes an exogenous thioesterase.

[0058] 40. The method of paragraph 35, wherein the cell is an *Escherichia* cell.

[0059] 41. The method of paragraph 35, wherein the cell produces higher levels of branched-chain fatty acids after expression of the polypeptide than it did prior to expression of the polypeptide.

[0060] 42. The method of paragraph 35, wherein the branched-chain fatty acids comprise one or more methyl branches.

[0061] 43. The method of paragraph 42, wherein the one or more methyl branches are on even numbered carbons.

[0062] 44. The method of paragraph 35, wherein the branched-chain fatty acids are not naturally produced in the cell.

[0063] 45. Branched-chain fatty acids produced by the method of paragraph 35.

[0064] 46. A cell comprising at least one recombinant polypeptide that increases the production of methylmalonyl-CoA in the cell, wherein the cell comprising the recombinant polypeptide produces more branched-chain fatty acids than an otherwise similar cell that does not comprise the recombinant polypeptide.

[0065] 47. A method for producing branched-chain fatty acids in a cell comprising: a. expressing in the cell a polypeptide that has propionyl-CoA synthetase activity; b. inhibiting propionylation of the propionyl-CoA synthetase; and c. culturing the cell under conditions suitable for producing the polypeptide, such that branched-chain fatty acids are produced.

[0066] 48. The method of paragraph 47, wherein the polypeptide does not include a lysine that is subject to propionylation.

[0067] 49. The method of paragraph 47, wherein step c) includes providing a source of resveratrol into a culture medium used to culture the cell.

[0068] 50. The method of paragraph 47, wherein the cell does not include an N-acetyltransferase enzyme responsible for propionylation of the propionyl-CoA synthetase.

[0069] 51. The method of paragraph 47, wherein the polypeptide has at least about 60% sequence identity to the protein encoded by SEQ ID NO: 22.

[0070] 52. The method of paragraph 47, wherein the cell contains increased enzymatic activity for removal of propionyl groups from one or more lysine residues of propionyl-CoA synthetase.

[0071] 53. The method of paragraph 47, wherein the method further includes expressing in the cell a polypeptide that encodes an exogenous thioesterase.

[0072] 54. The method of paragraph 47, wherein the cell is an *Escherichia* cell.

[0073] 55. The method of paragraph 47, wherein the cell produces higher levels of branched-chain fatty acids after expression of the polypeptide than it did prior to expression of the polypeptide.

[0074] 56. The method of paragraph 47, wherein the branched-chain fatty acids comprise one or more methyl branches.

[0075] 57. The method of paragraph 56, wherein the one or more methyl branches are on even numbered carbons.

[0076] 58. The method of paragraph 47, wherein the branched-chain fatty acids are not naturally produced in the cell.

[0077] 59. Branched-chain fatty acids produced by the method of paragraph 47.

[0078] 60. A method for producing branched-chain fatty acids in a cell comprising: a. expressing in the cell a polypeptide that has methylmalonyl-CoA mutase activity; b. expressing in a cell a polypeptide that has methylmalonyl-CoA epimerase activity; and c. culturing the cell under conditions suitable for producing the polypeptides, such that branched-chain fatty acids are produced.

[0079] 61. The method of paragraph 60, wherein the methylmalonyl-CoA mutase polypeptide has at least about 60% sequence identity to a sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 4.

[0080] 62. The method of paragraph 60, wherein the methylmalonyl-CoA epimerase polypeptide has at least about 60% sequence identity to a sequence set forth in SEQ ID NO: 6.

[0081] 63. The method of paragraph 60, wherein the method further includes expressing in the cell a polypeptide that encodes an exogenous thioesterase.

[0082] 64. The method of paragraph 60, wherein the cell is an *Escherichia* cell.

[0083] 65. The method of paragraph 60, wherein the cell produces higher levels of branched-chain fatty acids after expression of the polypeptide than it did prior to expression of the polypeptide.

[0084] 66. The method of paragraph 60, wherein the branched-chain fatty acids comprise one or more methyl branches.

[0085] 67. The method of paragraph 66, wherein the one or more methyl branches are on even numbered carbons.

[0086] 68. The method of paragraph 60, wherein the branched-chain fatty acids are not naturally produced in the cell.

[0087] 69. Branched-chain fatty acids produced by the method of paragraph 60.

[0088] 70. A cell comprising recombinant polypeptides having methylmalonyl-CoA mutase activity and methylmalonyl-CoA epimerase activity, wherein the cell comprising the recombinant polypeptides produces more branched-chain fatty acids than an otherwise similar cell that does not comprise the recombinant polypeptide.

[0089] 71. A composition comprising a mixture of biologically-produced branched-chain fatty acids, the branched-chain fatty acids having a chain length of C12 to C16 and from about 1 to about 3 methyl branches positioned on one or more even-numbered carbons.

[0090] 72. A method for producing branched-chain fatty acids in a cell comprising: a. expressing in the cell one or more recombinant polypeptides that increase the production of methylmalonyl-CoA in the cell; b. expressing in the cell a recombinant polypeptide that catalyzes the conversion of methylmalonyl-CoA to methylmalonyl-ACP; and c. culturing the cell under conditions suitable for producing the recombinant polypeptide, such that branched-chain fatty acids are produced.

[0091] 73. The method of paragraph 72, wherein the cell has a deletion in a gene for a methylmalonyl-CoA decarboxylase.

[0092] 74. The method of paragraph 72, wherein the cell additionally produces a recombinant polypeptide with a 3-ketoacyl-ACP synthase activity that recognizes methylmalonyl-ACP as a substrate.

[0093] 75. A method for producing branched-chain fatty acids in a cell comprising: a. expressing in the cell one or more recombinant polypeptides that increase the production of methylmalonyl-CoA in the cell; b. expressing in the cell a recombinant polypeptide that catalyzes the conversion of methylmalonyl-CoA to methylmalonyl-ACP; c. expressing in the cell a recombinant thioesterase; and d. culturing the cell under conditions suitable for producing the recombinant polypeptide, such that branched-chain fatty acids are produced.

[0094] 76. The method of paragraph 75, wherein the cell has a deletion in a gene for a methylmalonyl-CoA decarboxylase.

[0095] 77. The method of paragraph 75, wherein the cell additionally produces a recombinant polypeptide with a 3-ketoacyl-ACP synthase activity that recognizes methylmalonyl-ACP as a substrate.

[0096] 78. A method for producing branched-chain fatty acids in a cell, the branched-chain fatty acids having a chain length from about 10 to 18 carbons and branching at the second carbon, the method comprising: a. modifying the cell to increase carbon flow to methylmalonyl-CoA; and b. culturing the cell under conditions suitable for carbon flow to methylmalonyl-CoA to be increased, such that branched-chain fatty acids having a chain length from about 10 to about 18 carbons and branching at the second carbon are produced.

[0097] 79. The method of paragraph 78, wherein the branching at the second carbon is a methyl branch.

[0098] 80. A method for producing branched-chain fatty acids in a cell, the branched-chain fatty acids having a chain length from about 10 to 18 carbons and branching at the fourth carbon, the method comprising: a. modifying the cell to increase carbon flow to methylmalonyl-CoA; and b. culturing the cell under conditions suitable for carbon flow to methylmalonyl-CoA to be increased, such that branched-chain fatty acids having a chain length from about 10 to about 18 carbons and branching at the fourth carbon are produced.

[0099] 81. The method of paragraph 80, wherein the branching at the fourth carbon is a methyl branch.

[0100] 82. A method for producing branched-chain fatty acids in a cell, the branched-chain fatty acids having a chain length from about 10 to 18 carbons and branching at the sixth carbon, the method comprising: a. modifying the cell to

increase carbon flow to methylmalonyl-CoA; and b. culturing the cell under conditions suitable for carbon flow to methylmalonyl-CoA to be increased, such that branched-chain fatty acids having a chain length from about 10 to about 18 carbons and branching at the sixth carbon are produced.

[0101] 83. The method of paragraph 82, wherein the branching at the sixth carbon is a methyl branch.

[0102] 84. A method for producing branched-chain fatty acids in a cell, the branched-chain fatty acids having a chain length from about 12 to 18 carbons and branching at the eighth carbon, the method comprising: a. modifying the cell to increase carbon flow to methylmalonyl-CoA; and b. culturing the cell under conditions suitable for carbon flow to methylmalonyl-CoA to be increased, such that branched-chain fatty acids having a chain length from about 12 to about 18 carbons and branching at the eighth carbon are produced.

[0103] 85. The method of paragraph 84, wherein the branching at the eighth carbon is a methyl branch.

[0104] 86. A method for producing branched-chain fatty acids in a cell, the branched-chain fatty acids having a chain length from about 14 to 18 carbons and branching at the tenth carbon, the method comprising: a. modifying the cell to increase carbon flow to methylmalonyl-CoA; and b. culturing the cell under conditions suitable for carbon flow to methylmalonyl-CoA to be increased, such that branched-chain fatty acids having a chain length from about 14 to about 18 carbons and branching at the tenth carbon are produced.

[0105] 87. The method of paragraph 86, wherein the branching at the tenth carbon is a methyl branch.

[0106] 88. A method for producing branched-chain fatty acids in a cell, the branched-chain fatty acids having a chain length from about 16 to 18 carbons and branching at the twelfth carbon, the method comprising: a. modifying the cell to increase carbon flow to methylmalonyl-CoA; and b. culturing the cell under conditions suitable for carbon flow to methylmalonyl-CoA to be increased, such that branched-chain fatty acids having a chain length from about 16 to about 18 carbons and branching at the twelfth carbon are produced.

[0107] 89. The method of paragraph 88, wherein the branching at the twelfth carbon is a methyl branch.

[0108] 90. A method for producing branched-chain fatty acids in a cell, the branched-chain fatty acids having a chain length from about 10 to 18 carbons and branching at the second carbon, the method comprising: a. modifying the cell to generate methylmalonyl-ACP from methylmalonyl-CoA; and b. culturing the cell under conditions suitable for generation of methylmalonyl-ACP from methylmalonyl-CoA, such that branched-chain fatty acids having a chain length from about 10 to about 18 carbons and branching at the second carbon are produced.

[0109] 91. The method of paragraph 90, wherein the branching at the second carbon is a methyl branch.

[0110] 92. A method for producing branched-chain fatty acids in a cell, the branched-chain fatty acids having a chain length from about 10 to 18 carbons and branching at the fourth carbon, the method comprising: a. modifying the cell to generate methylmalonyl-ACP from methylmalonyl-CoA; and b. culturing the cell under conditions suitable for generation of methylmalonyl-ACP from methylmalonyl-CoA, such that branched-chain fatty acids having a chain length from about 10 to about 18 carbons and branching at the fourth carbon are produced.

[0111] 93. The method of paragraph 92, wherein the branching at the fourth carbon is a methyl branch.

[0112] 94. A method for producing branched-chain fatty acids in a cell, the branched-chain fatty acids having a chain length from about 10 to 18 carbons and branching at the sixth carbon, the method comprising: a. modifying the cell to generate methylmalonyl-ACP from methylmalonyl-CoA; and b. culturing the cell under conditions suitable for generation of methylmalonyl-ACP from methylmalonyl-CoA, such that branched-chain fatty acids having a chain length from about 10 to about 18 carbons and branching at the sixth carbon are produced.

[0113] 95. The method of paragraph 94, wherein the branching at the sixth carbon is a methyl branch.

[0114] 96. A method for producing branched-chain fatty acids in a cell, the branched-chain fatty acids having a chain length from about 12 to 18 carbons and branching at the eighth carbon, the method comprising: a. modifying the cell to generate methylmalonyl-ACP from methylmalonyl-CoA; and b. culturing the cell under conditions suitable for generation of methylmalonyl-ACP from methylmalonyl-CoA, such that branched-chain fatty acids having a chain length from about 12 to about 18 carbons and branching at the eighth carbon are produced.

[0115] 97. The method of paragraph 96, wherein the branching at the eighth carbon is a methyl branch.

[0116] 98. A method for producing branched-chain fatty acids in a cell, the branched-chain fatty acids having a chain length from about 14 to 18 carbons and branching at the tenth carbon, the method comprising: a. modifying the cell to generate methylmalonyl-ACP from methylmalonyl-CoA; and b. culturing the cell under conditions suitable for generation of methylmalonyl-ACP from methylmalonyl-CoA, such that branched-chain fatty acids having a chain length from about 14 to about 18 carbons and branching at the tenth carbon are produced.

[0117] 99. The method of paragraph 98, wherein the branching at the tenth carbon is a methyl branch.

[0118] 100. A method for producing branched-chain fatty acids in a cell, the branched-chain fatty acids having a chain length from about 16 to 18 carbons and branching at the twelfth carbon, the method comprising: a. modifying the cell to generate methylmalonyl-ACP from methylmalonyl-CoA; and b. culturing the cell under conditions suitable for generation of methylmalonyl-ACP from methylmalonyl-CoA, such that branched-chain fatty acids having a chain length from about 16 to about 18 carbons and branching at the twelfth carbon are produced.

[0119] 101. The method of paragraph 100, wherein the branching at the twelfth carbon is a methyl branch.

[0120] 102. A method for producing modified fatty acids in a cell comprising: a. providing a cell having type II fatty acid synthase activity; b. expressing in the cell one or more recombinant polypeptides that catalyze formation of at least one intermediate metabolite, wherein the at least one intermediate metabolite is incorporated by the type II fatty acid synthase; and c. culturing the cell under conditions suitable for producing the recombinant polypeptide, such that modified fatty acids are produced.

[0121] 103. The method of paragraph 102, wherein the cell is an *Escherichia* cell.

[0122] 104. The method of paragraph 102, wherein the intermediate metabolite is methylmalonyl-ACP.

[0123] 105. The method of paragraph 102, wherein the polypeptide(s) catalyze the conversion of methylmalonyl-CoA to methylmalonyl-ACP.

[0124] 106. The method of paragraph 102, wherein the cell produces higher levels of modified fatty acids after expression of the polypeptide than it did prior to expression of the polypeptide.

[0125] 107. The method of paragraph 102, wherein the modified fatty acids comprise one or more methyl branches on even-numbered carbons.

[0126] 108. The method of paragraph 102, wherein the polypeptide is an acyl transferase.

[0127] 109. The method of paragraph 102, wherein the polypeptide is encoded by fabD.

[0128] 110. The method of paragraph 102, wherein the polypeptide is a polyketide synthase or a portion thereof.

[0129] 111. The method of paragraph 102, wherein the polypeptide is a *Mycobacterium* mycocerosic acid synthase or a portion thereof.

[0130] 112. An *Escherichia* cell that produces branched-chain fatty acids having a chain length from about 10 to about 18 carbons and comprising one or more methyl branches on one or more even-numbered carbons.

BRIEF DESCRIPTION OF THE DRAWINGS

[0131] FIG. **1** is a mutA nucleotide sequence (SEQ ID NO: 1).

[0132] FIG. **2** is a mutB nucleotide sequence (SEQ ID NO: 2).

[0133] FIG. **3** is a MutA protein sequence (SEQ ID NO: 3).

[0134] FIG. **4** is a MutB protein sequence (SEQ ID NO: 4).

[0135] FIG. **5** is a methylmalonyl-CoA epimerase nucleotide sequence (SEQ ID NO: 5).

[0136] FIG. **6** is a methylmalonyl-CoA epimerase protein sequence (SEQ ID NO: 6).

[0137] FIG. **7** is a DNA sequence for accA1 (GenBank Accession No. AF113603.1) (SEQ ID NO: 7).

[0138] FIG. **8** is a DNA sequence for pccB (GenBank Accession No. AF113605.1) (SEQ ID NO: 8).

[0139] FIG. **9** is a protein sequence for AccA1 (SEQ ID NO: 9).

[0140] FIG. **10** is a protein sequence for PccB (SEQ ID NO: 10).

[0141] FIG. **11** shows element 1 including the $P_{Llac0\text{-}1}$ sequence and the phage T7 gene10 ribosome binding site (SEQ ID NO: 11).

[0142] FIG. **12** shows element 2 including the optimized accA1 gene sequence (SEQ ID NO: 12).

[0143] FIG. **13** shows element 3 including the spacer sequence (SEQ ID NO: 13).

[0144] FIG. **14** shows element 4 including the optimized pccB sequence (SEQ ID NO: 14).

[0145] FIG. **15** is a synthetic sequence for propionyl-CoA carboxylase gene expression (SEQ ID NO: 15).

[0146] FIG. **16** is the forward primer sequence for PrpE (SEQ ID NO: 16).

[0147] FIG. **17** is the reverse primer sequence for PrpE (SEQ ID NO: 17).

[0148] FIG. **18** is the MMAT domain sequence from *Mycobacterium bovis* BCG (SEQ ID NO: 18).

[0149] FIG. **19** is a protein sequence for the *Mycobacterium bovis* BCG MAS (GenBank Accession No. YP_979046) (SEQ ID NO: 19).

[0150] FIG. **20** is a codon-optimized MMAT domain DNA sequence from *Mycobacterium bovis* BCG (SEQ ID NO: 20).

[0151] FIG. **21** is an alignment of a codon-optimized MMAT domain from *Mycobacterium bovis* BCG with the original sequence (SEQ ID NOs: 20 and 21).

[0152] FIG. **22** is the protein sequence of *Salmonella enterica* propionyl CoA synthase PrpE (GenBank Accession No. AAC44817) (SEQ ID NO: 22).

[0153] FIG. **23** is the DNA sequence of *Salmonella enterica* propionyl CoA synthase PrpE (SEQ ID NO. 23).

[0154] FIG. **24** is a bar graph illustrating methylmalonyl-CoA production (ng/ml) in *E. coli* strain K27-Z1 harboring pTrcHisA pZA31 (control), pZA31 mutAB Ss epi (MutAB Epi), pTrcHisA Ec sbm (Sbm), or pTrcHisA Ec sbm pZA31 Mb mmat (Sbm/Mmat). No methylmalonyl-CoA was identified in the control sample; the figure indicates the background level of detection.

[0155] FIG. **25** is a bar graph illustrating methylmalonyl-CoA production (ng/ml) in *E. coli* BW25113 (control) and BW25113 harboring pZA31-accA1-pccB (Pcc). No methylmalonyl-CoA was identified in the control sample; the figure indicates the background level of detection. Two biological replicates are represented.

[0156] FIG. **26** is a two-dimensional (2D) representation of the 2D Total Ion Chromatogram resulting from a sample of fatty acid produced by BL21 Star (DE3) *E. coli* harboring pTrcHisA Ec sbm So ce epi pZA31 mmat. Light areas on the figure indicate the presence of sample material. Peak names and arrows indicate samples that were further characterized by mass spectrometry.

[0157] FIG. **27** is a two-dimensional (2D) representation of the 2D Total Ion Chromatogram resulting from a sample produced by a control strain, BL21 Star (DE3) *E. coli* harboring pTrcHisA pZA31. No branched-chain fatty acid was detected. Arrows indicate the presence of straight-chain fatty acid derivatives of the indicated chain length.

[0158] FIG. **28** is a representation of the mass spectra of peaks 54, 55, and 57 identified in FIG. **26**. Eight- and ten-carbon branched-chain fatty acids are depicted in the top two profiles and were identified by the almost complete absence of the circled fragment. A twelve-branched fatty acid was tentatively identified and is depicted in the third profile.

DETAILED DESCRIPTION OF THE INVENTION

[0159] The invention relates to improved biological production of scattered branched-chain fatty acids. In addition, in certain embodiments, the invention provides improved compositions of biologically produced scattered branched-chain fatty acids having defined chain lengths with methyl branches at one or more even-numbered carbons within the fatty acid. In addition, in certain embodiments, the fatty acid length can be tailored to a predetermined length, such as, for example, to produce fatty acids with a backbone of C12 to C16. In certain embodiments, the methods and/or cells can produce a mixture of fatty acids having varied numbers of methyl branches, varied positions of the methyl branches, and varied length of the fatty acids, such as, for example, a mixture of fatty acids having a chain length of C12 to C16 and from about 0 to about 3 methyl branches positioned on one or more even-numbered carbons.

[0160] As used herein, "amplify," "amplified," or "amplification" refers to any process or protocol for copying a polynucleotide sequence into a larger number of polynucleotide molecules, e.g., by reverse transcription, polymerase chain reaction, and ligase chain reaction.

[0161] As used herein, an "antisense sequence" refers to a sequence that specifically hybridizes with a second polynucleotide sequence. For instance, an antisense sequence is a DNA sequence that is inverted relative to its normal orientation for transcription. Antisense sequences can express an RNA transcript that is complementary to a target mRNA molecule expressed within the host cell (e.g., it can hybridize to target mRNA molecule through Watson-Crick base pairing).

[0162] As used herein, "cDNA" refers to a DNA that is complementary or identical to an mRNA, in either single stranded or double stranded form.

[0163] As used herein, the carbons in fatty acids are numbered with the first carbon as part of the carboxylic acid group, and the second carbon (C2) adjacent to the first. The numbers continue so that the highest number carbon is farthest from the carboxylic acid group. "Even number" carbons include C2, C4, C6, C8, C10, C12, C14, and so on.

[0164] As used herein, "complementary" refers to a polynucleotide that can base pair with a second polynucleotide. Put another way, "complementary" describes the relationship between two single-stranded nucleic acid sequences that anneal by base-pairing. For example, a polynucleotide having the sequence 5'-GTCCGA-3' is complementary to a polynucleotide with the sequence 5'-TCGGAC-3'.

[0165] As used herein, a "conservative substitution" refers to the substitution in a polypeptide of an amino acid with a functionally similar amino acid. Put another way, a conservative substitution involves replacement of an amino acid residue with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined within the art, and include amino acids with basic side chains (e.g., lysine, arginine, and histidine), acidic side chains (e.g., aspartic acid and glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, and cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, and tryptophan), beta-branched side chains (e.g., threonine, valine, and isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, and histidine).

[0166] As used herein, "encoding" refers to the inherent property of nucleotides to serve as templates for synthesis of other polymers and macromolecules. Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence.

[0167] As used herein, "endogenous" refers to polynucleotides, polypeptides, or other compounds that are expressed naturally or originate within an organism or cell. That is, endogenous polynucleotides, polypeptides, or other compounds are not exogenous. For instance, an "endogenous" polynucleotide or peptide is present in the cell when the cell was originally isolated from nature.

[0168] As used herein, "expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. For example, suitable expression vectors include, without limitation, autonomously replicating vectors or vectors integrated into the chromosome. In some instances, an expression vector is a viral-based vector.

[0169] As used herein, "exogenous" refers to any polynucleotide or polypeptide that is not naturally expressed or produced in the particular cell or organism where expression is desired. Exogenous polynucleotides, polypeptides, or other compounds are not endogenous.

[0170] As used herein, "hybridization" includes any process by which a strand of a nucleic acid joins with a complementary nucleic acid strand through base-pairing. Thus, the term refers to the ability of the complement of the target sequence to bind to a test (i.e., target) sequence, or vice-versa.

[0171] As used herein, "hybridization conditions" are typically classified by degree of "stringency" of the conditions under which hybridization is measured. The degree of stringency can be based, for example, on the melting temperature (T_m) of the nucleic acid binding complex or probe. For example, "maximum stringency" typically occurs at about T_m-5° C. (5° below the T_m of the probe); "high stringency" at about 5-10° C. below the T_m; "intermediate stringency" at about 10-20° below the T_m of the probe; and "low stringency" at about 20-25° C. below the T. Alternatively, or in addition, hybridization conditions can be based upon the salt or ionic strength conditions of hybridization and/or one or more stringency washes. For example, 6×SSC=very low stringency; 3×SSC=low to medium stringency; 1×SSC=medium stringency; and 0.5×SSC=high stringency. Functionally, maximum stringency conditions may be used to identify nucleic acid sequences having strict (i.e., about 100%) identity or near-strict identity with the hybridization probe; while high stringency conditions are used to identify nucleic acid sequences having about 80% or more sequence identity with the probe.

[0172] As used herein, "identical" or percent "identity" in the context of two or more polynucleotide or polypeptide sequences refers to two or more sequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using sequence comparison algorithms or by visual inspection.

[0173] As used herein, "long-chain fatty acids" refers to fatty acids with aliphatic tails longer than 14 carbons. In some embodiments of the invention, long-chain fatty acids are provided that comprise 15, 16, 17, 18, 19, 20, 21, or 22 carbons in the carbon backbone.

[0174] As used herein, "medium-chain fatty acids" refers to fatty acids with aliphatic tails between 6 and 14 carbons. In certain embodiments, the medium-chain fatty acids can have from 11 to 13 carbons.

[0175] As used herein, "naturally-occurring" refers to an object that can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

[0176] As used herein, "operably linked," when describing the relationship between two DNA regions or two polypeptide regions, means that the regions are functionally related to each other. For example, a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation; and a signal sequence is operably linked to a peptide if it functions as a signal sequence, such as by participating in the secretion of the mature form of the protein.

[0177] As used herein, "overexpression" refers to expression of a polynucleotide to produce a product (e.g., a polypeptide or RNA) at a higher level than the polynucleotide is

normally expressed in the host cell. An overexpressed polynucleotide is generally a polynucleotide native to the host cell, the product of which is generated in a greater amount than that normally found in the host cell. Overexpression is achieved by, for instance and without limitation, operably linking the polynucleotide to a different promoter than the polynucleotide's native promoter or introducing additional copies of the polynucleotide into the host cell.

[0178] As used herein, "polynucleotide" refers to a polymer composed of nucleotides. The polynucleotide may be in the form of a separate fragment or as a component of a larger nucleotide sequence construct, which has been derived from a nucleotide sequence isolated at least once in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard molecular biology methods, for example, using a cloning vector. When a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T." Put another way, "polynucleotide" refers to a polymer of nucleotides removed from other nucleotides (a separate fragment or entity) or can be a component or element of a larger nucleotide construct, such as an expression vector or a polycistronic sequence. Polynucleotides include DNA, RNA and cDNA sequences.

[0179] As used herein, "polypeptide" refers to a polymer composed of amino acid residues which may or may not contain modifications such as phosphates and formyl groups.

[0180] As used herein, "recombinant expression vector" refers to a DNA construct used to express a polynucleotide that encodes a desired polypeptide. A recombinant expression vector can include, for example, a transcriptional subunit comprising (i) an assembly of genetic elements having a regulatory role in gene expression, for example, promoters and enhancers, (ii) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (iii) appropriate transcription and translation initiation and termination sequences. Recombinant expression vectors are constructed in any suitable manner. The nature of the vector is not critical, and any vector may be used, including plasmid, virus, bacteriophage, and transposon. Possible vectors for use in the invention include, but are not limited to, chromosomal, non-chromosomal and synthetic DNA sequences, e.g., bacterial plasmids; phage DNA; yeast plasmids; and vectors derived from combinations of plasmids and phage DNA, DNA from viruses such as vaccinia, adenovirus, fowl pox, baculovirus, SV40, and pseudorabies.

[0181] As used herein, "primer" refers to a polynucleotide that is capable of specifically hybridizing to a designated polynucleotide template and providing a point of initiation for synthesis of a complementary polynucleotide when the polynucleotide primer is placed under conditions in which synthesis is induced.

[0182] As used herein, "recombinant polynucleotide" refers to a polynucleotide having sequences that are not naturally joined together. A recombinant polynucleotide may be included in a suitable vector, and the vector can be used to transform a suitable host cell. A host cell that comprises the recombinant polynucleotide is referred to as a "recombinant host cell." The polynucleotide is then expressed in the recombinant host cell to produce, e.g., a "recombinant polypeptide."

[0183] As used herein, "specific hybridization" refers to the binding, duplexing, or hybridizing of a polynucleotide preferentially to a particular nucleotide sequence under stringent conditions.

[0184] As used herein, "stringent conditions" refers to conditions under which a probe will hybridize preferentially to its target subsequence, and to a lesser extent to, or not at all to, other sequences.

[0185] As used herein, "short-chain fatty acids" refers to fatty acids having aliphatic tails with fewer than 6 carbons.

[0186] As used herein, "substantially homologous" or "substantially identical" in the context of two nucleic acids or polypeptides, generally refers to two or more sequences or subsequences that have at least 40%, 60%, 80%, 90%, 95%, 96%, 97%, 98% or 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using sequence comparison algorithms or by visual inspection. The substantial identity can exist over any suitable region of the sequences, such as, for example, a region that is at least about 50 residues in length, a region that is at least about 100 residues, or a region that is at least about 150 residues. In certain embodiments, the sequences are substantially identical over the entire length of either or both comparison biopolymers.

[0187] In one embodiment, the invention relates to a novel method of producing scattered branched-chain fatty acids (or products derived from scattered branched-chain fatty acid) using bacteria. In general, the method includes increasing the supply of methylmalonyl-CoA and/or the conversion of methylmalonyl-CoA to methylmalonyl-ACP within the cell, incorporating the branch from the methylmalonyl-CoA into the fatty acid, and, optionally, using a thioesterase to specify the range of size of the fatty acids. In certain embodiments, the method provides branched-chain fatty acids having a chain length of C12 to C16. In addition, in certain embodiments, the branched-chain fatty acids have from about 0 to about 3 methyl branches, such as from about 1 to about 3 methyl branches, such as, for example, from about 1 to about 2 methyl branches, or 1, 2, or 3 methyl branches positioned on one or more carbons. In certain embodiments, the methyl branches are positioned on even-numbered carbons.

[0188] In one embodiment, scattered branched-chain fatty acid production is increased by increasing the production of methylmalonyl-CoA within the cell via, e.g., propionyl-CoA and/or succinyl-CoA intermediates. Thus, in one aspect, the invention provides a method for producing branched-chain fatty acid comprising a methyl on one or more even number carbons. The method comprises culturing a cell comprising an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a polypeptide that catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA and/or an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a polypeptide that catalyzes the conversion of succinyl-CoA to methylmalonyl-CoA. The cell is cultured under conditions allowing expression of the polynucleotide(s) and production of the branched-chain fatty acid. The cell produces more branched-chain fatty acid comprising a methyl branch on one or more even number carbons than an otherwise similar cell that does not comprise the polynucleotide(s) (e.g., a cell of the same cell type or derived from the same organism that does not comprise the polynucleotide(s)). Propionyl-CoA is converted to methylmalonyl-CoA by, e.g., the action of a propionyl-CoA carboxylase. Any propionyl-CoA carboxylase that catalyzes the

conversion of propionyl-CoA to methylmalonyl-CoA is suitable for use in the inventive method. An exemplary propionyl-CoA carboxylase is a carboxylase from *Streptomyces coelicolor*, which comprises two heterologous subunits encoded by pccB and by either accA1 or accA2. In certain embodiments, the cell of the inventive method is engineered to produce PccB and AccA1 or PccB and AccA2. In one aspect, the cell comprises one or more polynucleotides encoding polypeptide(s) comprising an amino acid sequence at least about 80% identical (e.g., 85%, 90%, 95%, or 100% identical) to the amino acid sequences set forth in SEQ ID NO: 9 and/or 10. Additional, non-limiting examples of polypeptides that catalyze the conversion of propionyl-CoA to methylmalonyl-CoA are propionyl-CoA carboxylases from *Mycobacterium smegmatis, Homo sapiens, Acinetobacter baumannii, Brucella suis, Saccharopolyspora erythraea, Burkholderia glumae*, and *Aedes aegypti*, as well as the propionyl-CoA carboxylases set forth in Table A.

TABLE A

Organism	GenBank Accession	Description	SEQ ID NO:
Ehrlichia chaffeensis	YP_507303	Propionyl-CoA carboxylase alpha subunit (PCCA)	51
Ehrlichia chaffeensis	YP_507410	Propionyl-CoA carboxylase beta subunit (PCCB)	52
Agrobacterium vitis	YP_002547482	Propionyl-CoA carboxylase alpha subunit (PCCA)	53
Agrobacterium vitis	YP_002547479	Propionyl-CoA carboxylase beta subunit (PCCB)	54
Methylobacterium extorquens	YP_003069256	Propionyl-CoA carboxylase alpha subunit (PCCA)	55
Methylobacterium extorquens	YP_003065890	Propionyl-CoA carboxylase beta subunit (PCCB)	56
Sinorhizobium meliloti	NP_437988	Propionyl-CoA carboxylase alpha subunit (PCCA)	57
Sinorhizobium meliloti	NP_437987	Propionyl-CoA carboxylase beta subunit (PCCB)	58
Ruegeria pomeroyi	YP_166352	Propionyl-CoA carboxylase alpha subunit (PCCA)	59
Ruegeria pomeroyi	YP_166345	Propionyl-CoA carboxylase beta subunit (PCCB)	60

[0189] Optionally, the cell is modified to increase carbon flow to propionyl-CoA (and then onward to methylmalonyl-CoA) by, for example, increasing expression of (i.e., overexpressing) prpE or other propionyl-CoA synthetase genes. Alternatively or in addition, an exogenous polynucleotide comprising a nucleic acid sequence encoding a propionyl-CoA synthetase is introduced into the host cell to upregulate propionyl-CoA production. Additionally, feeding host cells (e.g., microbes) large amounts of methionine, isoleucine, valine, threonine, propionic acid, and/or odd-chain length fatty acids (such as valeric acid) increases production of the propionyl-CoA precursor of methylmalonyl-CoA.

[0190] Methylmalonyl-CoA production via propionyl-CoA also is increased utilizing the metabolic pathway that converts pyruvate to propionyl-CoA, with lactate, lactoyl-CoA, and acrylyl-CoA as intermediates. Carbon flow to propionyl-CoA is upregulated by overproducing the enzymes of the pathway, producing exogenous enzymes catalyzing one or more conversions of the pathway, and/or by providing pyruvate or lactate in larger amounts than normally found in the host cell. For example, in any embodiment of the invention, the cell comprises an exogenous or overexpressed polynucleotide encoding lactate dehydrogenase, lactate CoA transferase, lactyl-CoA dehydratase, and/or acrylyl-CoA reductase.

[0191] In addition, in any aspect of the invention, carbon flow to branch pathways not contributing to formation of the desired branched-chain fatty acid is minimized by attenuation of endogenous enzyme activity responsible for the diversion of carbon. Complete abolishment of endogenous activity is not required; any reduction in activity is suitable in the context of the invention. Enzyme activity is attenuated (i.e., reduced or abolished) by, for example, mutating the coding sequence for the enzyme to create a non-functional or reduced-function polypeptide, by removing all or part of the coding sequence for the enzyme from the cellular genome, by interfering with translation of an RNA transcript encoding the enzyme (e.g., using antisense oligonucleotides), or by manipulating the expression control sequences influencing expression of the enzyme. For example, in one aspect, the cell is modified to prevent methylmalonyl-CoA degradation, thereby increasing the amount of methylmalonyl-CoA available for conversion to methylmalonyl-ACP. Methylmalonyl-CoA degradation is reduced by, for example, deleting or inactivating methylmalonyl-CoA decarboxylase from the host. Put another way, the cell is modified to attenuate endogenous methylmalonyl-CoA decarboxylase activity. In *E. coli*, for example, methylmalonyl-CoA decarboxylase activity is attenuated by, for example, deleting or mutating ygfG. Optionally, endogenous acyl transferase activity is attenuated. Alternatively or in addition, methylmalonyl-CoA production within the cell is increased by preventing alternative metabolism of propionyl-CoA to succinyl-CoA, such as, for example, by deleting or otherwise reducing (attenuating) the activity of an endogenous methylmalonyl-CoA mutase gene. Optionally, methylmalonyl-CoA levels are increased by increasing the degradation of valine directly to methylmalonyl-CoA. Valine degradation comprises the following intermediates: α-ketoisovalerate, isobutyryl-CoA, methacrylyl-CoA, β-hydroxyisobutyryl-CoA, β-hydroxyisobutyrate, and methylmalonate semialdehyde. Optionally, methylmalonate semialdehyde is converted directly to methylmalonyl-CoA or

indirectly through a propionyl-CoA intermediate. In an exemplary embodiment, the cell of the invention comprises an overexpressed or exogenous polynucleotide comprising a nucleic acid sequence encoding one or more of the following enzymes: L-valine:2-oxoglutarate aminotransferase, 2-oxoisovalerate dehydrogenase, isobutyryl-CoA:FAD oxidoreductase, 3-hydroxy-isobutyryl-CoA hydro-lyase, 3-hydroxyisobutyryl-CoA hydrolase, 3-hydroxyisobutyrate dehydrogenase, and/or methylmalonate-semialdehyde dehydrogenase. Methylmalonate-semialdehyde dehydrogenase catalyzes the production of propanoyl-CoA, which can be converted to methylmalonyl-CoA by propanoyl-CoA carboxylase.

[0192] In one aspect, the cell comprises an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a polypeptide that catalyzes the conversion of succinyl-CoA to methylmalonyl-CoA. An exemplary polypeptide that catalyzes the reaction is methylmalonyl-CoA mutase. In any embodiment of the invention, the cell is engineered to overexpress a methylmalonyl-CoA mutase gene, such as, for example, sbm (encoding Sleeping Beauty mutase) in *E. coli*. Alternatively or in addition, an exogenous polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA mutase is expressed in the cell. Exemplary methylmalonyl-CoA mutases include, but are not limited to, Sbm from *E. coli*, MutA and/or MutB from *Streptomyces cinnamonensis*, and methylmalonyl-CoA mutases from *Janibacter* sp. HTCC2649, *Corynebacterium glutamicum, Euglena gracilis, Homo sapiens, Propionibacterium shermanii, Bacillus megaterium*, and *Mycobacterium smegmatis*. Additional, non-limiting examples of polypeptides that catalyze the conversion of succinyl-CoA to methylmalonyl-CoA are provided in Table B.

TABLE B

Organism	GenBank Accession	Description	SEQ ID NO.
Bacillus megaterium	YP_003564880	methylmalonyl-CoA mutase small subunit (mutA)	61
Bacillus megaterium	YP_003564879	methylmalonyl-CoA mutase large subunit (mutB)	62
Mycobacterium tuberculosis	YP_001282809	methylmalonyl-CoA mutase small subunit (mutA)	63
Mycobacterium tuberculosis	YP_001282810	methylmalonyl-CoA mutase large subunit (mutB)	64
Corynebacterium glutamicum	YP_225814	methylmalonyl-COA mutase small subunit (mutA)	65
Corynebacterium glutamicum	YP_225813	methylmalonyl-CoA mutase large subunit (mutB)	66
Rhodococcus erythropolis	YP_002766535	methylmalonyl-CoA mutase small subunit (mutA)	67
Rhodococcus erythropolis	YP_002766536	methylmalonyl-CoA mutase large subunit (mutB)	68
Porphyromonas gingivalis	NP_905776	methylmalonyl-CoA mutase small subunit (mutA)	69
Porphyromonas gingivalis	NP_905777	methylmalonyl-CoA mutase large subunit (mutB)	70

[0193] In one aspect, the cell comprises one or more polynucleotides encoding polypeptide(s) comprising an amino acid sequence at least about 80% identical (e.g., 85%, 90%, 95%, or 100% identical) to the amino acid sequences set forth in SEQ ID NO: 3, 4, and/or 28. The cell can comprise polynucleotides encoding a methylmalonyl-CoA mutase, a propionyl-CoA carboxylase, or both.

[0194] Depending on the substrate specificity of the fatty acid synthase produced by the cell, a methylmalonyl-CoA epimerase also may be desired to facilitate use of methylmalonyl-CoA as a precursor in fatty acid synthesis. Thus, in one aspect, the cell further comprises an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA epimerase. Methylmalonyl-CoA epimerases suitable for use in the invention include, but are not limited to, *Sorangium cellulosum* So ce 56 methylmalonyl-CoA epimerase, *Streptomyces sviceus* ATCC 29083 methylmalonyl-CoA epimerase, *Kribbella flavida* DSM 17836 methylmalonyl-CoA epimerase, and methylmalonyl-CoA epimerases from *Homo sapiens, Bacillus megaterium*, and *Mycobacterium smegmatis*.

[0195] Production of branched-chain fatty acid comprising a methyl branch on one or more even number carbons also is enhanced by upregulating conversion of methylmalonyl-CoA to methylmalonyl-ACP. In one or more embodiments, conversion of methylmalonyl-CoA to methylmalonyl-ACP is increased in the cell by engineering the cell to produce an acyl transferase (such as the acyl transferase encoded by fabD in *E. coli*) to catalyze the formation of methylmalonyl-ACP from methylmalonyl-CoA. Put another way, in one aspect, the cell further comprises an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding an acyl transferase. Any suitable acyl transferase can be used, such as, for example and without limitation, an acyl transferase domain from a polyketide synthase, such as those involved in the synthesis of monensin, epothilone, amphotericin, candicidin, nystatin, pimaricin, ascomycin, rapamycin, avermiectin, spinosad, mycinamicin, niddamycin, oleandomycin, megalomicin, nanchangmycin, picromycin, rifamycin, oligomycin erythromycin, polyenes, and macrolides, and an acyl transferase domain from *Mycobacterium* mycocerosic acid synthase. Acyl transferase domains from larger fatty acid synthase enzymes, such as *Mycobacterium* mycocerosic acid synthase, act upon methylmalonyl-CoA in the absence of other enzymatic domains of the larger synthase. Optionally, the acyl transferase lacks polyketide synthesis activity. By "polyketide synthesis activity" is meant enzymatic activity,

other than acyl transferase activity, that catalyzes the production of polyketides in a host cell, such as, for example and without limitation, acyltransferase activity, ketoacyl synthase activity, ketoacyl reductase activity, dehydratase activity, enoyl reductase activity, acyl carrier protein activity, and thioesterase activity.

[0196] Alternatively, or in addition, in certain embodiments, a 3-ketoacyl-ACP synthase domain, such as, for example, a domain from a polyketide synthase or a mycocerosic acid synthase, is added to the fatty acid synthase of the host cell. In certain embodiments, the host cell (e.g., microbe) is engineered to include both acyl transferase and 3-ketoacyl-ACP synthase domains that can recognize methylmalonyl-CoA. In addition, in certain embodiments, genes for the endogenous acyl transferase and/or 3-ketoacyl-ACP synthase activities can be attenuated (e.g., deleted) to minimize the amount of malonyl-CoA incorporation in fatty acid synthesis.

[0197] In certain embodiments, the invention includes use of a thioesterase to specify the chain length of the fatty acid, such as, for example, to produce medium-chain fatty acids. In certain embodiments, the host cell further comprises an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a thioesterase. In one aspect, the host cell (e.g., bacteria) is engineered to produce a thioesterase that assists in the production of medium-chain branched-chain fatty acids. Alternatively, the host cell is engineered to produce (or overproduce) a thioesterase that assists in the production of long-chain branched-chain fatty acids. Exemplary thioesterases include, for example, the mallard uropygial gland thioesterase, the California bay thioesterase, the rat mammary gland thioesterase II, *E. coli* TesA, the *Cuphea wrightii* thioesterase, and other thioesterases suitable for production of the desired chain-length fatty acids.

[0198] Optionally, the cell is modified to produce (or increase the production of) branched acyl-CoA, which is a substrate for elongase in the production of long chain fatty acid. In this regard, in an exemplary embodiment of the invention, the cell comprises an exogenous or overexpressed polynucleotide comprising a nucleic acid encoding a coenzyme-A synthetase, which converts branched-chain fatty acid to branched acyl-CoA. Examples of coenzyme-A synthetases include, but are not limited to, the coenzyme-A synthetase from *Leishmania braziliensis* (GenBank Accession No. XP_001561614), and the coenzyme-A synthetase from *Escherichia coli* (GenBank Accession No. YP_541006). Optionally, the cell comprises exogenous or overexpressed polynucleotide(s) comprising a nucleic acid sequence encoding an elongase to increase the length of the carbon backbone. Elongases are enzyme complexes that exhibit 3-ketoacyl-CoA synthase, 3-ketoacyl-CoA reductase, 3-hydroxyacyl-CoA dehydratase, and enoyl-CoA reductase activities, and generally utilize malonyl-CoA as an extension unit for extending the carbon chain. When a methyl-malonyl CoA is used as an extension unit by the enzyme complex, additional methyl branches are introduced at even carbon positions. Exemplary elongases include, but are not limited to, elongases comprising the one or more of the following subunits: *Saccharomyces cerevisiae* 3-ketoacyl-CoA synthase (GenBank Accession No. NP_013476), 3-ketoacyl-CoA reductase (GenBank Accession No. NP_009717), 3-hydroxyacyl-CoA dehydratase (GenBank Accession No. NP_012438) and enoyl-CoA reductase (GenBank Accession No. NP_010269); and *Arabidopsis thaliana* col 3-ketoacyl-CoA synthase (GenBank Accession No. NP_849861), 3-ketoacyl-CoA reductase (GenBank Accession No. NP_564905), 3-hydroxyacyl-CoA dehydratase (GenBank Accession No. NP_193180), and enoyl-CoA reductase (GenBank Accession No. NP_191096).

[0199] Any suitable cell or organism, such as, for example, bacterial cells and other prokaryotic cells, and yeast cells, can be used in the context of the invention. In one aspect, the invention relates to cells, such as *Escherichia* cells (e.g., *E. coli*), which naturally produce Type II fatty acid synthase and/or do not naturally produce scattered branched-chain fatty acid (i.e., branched-chain fatty acid comprising a methyl branch on one or more even numbered carbons). These cells are engineered to produce the branched-chain fatty acids as described herein. Alternatively, the cell naturally produces branched-chain fatty acid and is modified as described herein to produce higher levels of branched-chain fatty acid (or different proportions of different types of branched-chain fatty acid) compared to an unmodified cell. In certain embodiments, fatty acid is manufactured using bacteria known to make the methylmalonyl-CoA precursor, such as *Streptomyces, Mycobacterium* or *Corynebacterium*. These bacteria are, in one aspect, engineered to produce (i) an acyl transferase to increase carbon flux to methylmalonyl-ACP that is incorporated in the fatty acid synthesis pathway and/or (ii) a thioesterase to control the chain length.

[0200] Exemplary bacteria that are suitable for use in the invention include, but are not limited to, *Spirochaeta aurantia, Spirochaeta littoralis, Pseudomonas maltophilia, Pseudomonas putrefaciens, Xanthomonas campestris, Legionella anisa, Moraxella catarrhalis, Thermus aquaticus, Flavobacterium aquatile, Bacteroides asaccharolyticus, Bacteroides fragilis, Succinimonas amylolytica, Desulfovibrio africanus, Micrococcus agilis, Stomatococcus mucilaginosus, Planococcus citreus, Marinococcus albusb, Staphylococcus aureus, Peptostreptococcus anaerobius, Ruminococcus albus, Sarcina lutea, Sporolactobacillus inulinus, Clostridium thermocellum, Sporosarcina ureae, Desulfotomaculum nigrificans, Listeria monocytogenes, Brochothrix thermosphacta, Renibacterium salmoninarum, Kurthia zopfii, Corynebacterium aquaticum, Arthrobacter radiotolerans, Brevibacterium fermentans, Propionibacterium acidipropionici, Eubacterium lentum, Cytophaga aquatilis, Sphingobacteriuma multivorumb, Capnocytophaga gingivalis, Sporocytophaga myxococcoides, Flexibacter elegans, Myxococcus coralloides, Archangium gephyra, Stigmatella aurantiaca, Oerskovia turbata, Escherichia coli, Bacillus subtilis, Salmonella typhimurium, Corynebacterium glutamicum, Streptomyces coelicolor, Streptomyces lividans, Clostridium thermocellum* and *Saccharomonospora viridis.*

[0201] In one aspect, the fatty acid produced by the inventive cell comprises about 80% to about 100% (wt.) (e.g., about 85%, about 90%, or about 95%) linear and branched-chain fatty acid. Of the linear and branched-chain fatty acids produced by the cell, approximately 1% to approximately 95% or more (e.g., 5%, 10%, 15%, 20%, 30%, 50%, 60%, 75%, 85%, or 100%) is branched-chain fatty acid comprising a methyl group on one or more even carbons. In some embodiments, the cell does not produce, or produces only trace amounts of, fatty acid comprising methyl branching on odd numbered carbons. By "trace amount" is meant less than 1% of the total fatty acid content produced by the cell. Alternatively or in addition, in one aspect, the mixture of fatty acids produced by the cell comprises no more than 50% end-terminal-branched fatty acid (i.e., fatty acids that contain branch-

ing on a carbon atom that is within 40% of the non-functionalized terminus of the longest carbon chain). Optionally, the inventive cell is modified to preferentially produce branched-chain fatty acid with desired chain lengths, e.g., about six to about 18 carbons or more in the carbon backbone (not including the methyl branch(es)). In some embodiments, the host cell preferentially generates long chain fatty acid, medium-length chain fatty acid, short chain fatty acid, or a desired combination fatty acids (e.g., 60%, 70%, 80%, 85%, 90%, 95% or more of the branched-chain fatty acid produced by the cell comprises the desired number of carbons). In addition, in certain embodiments, the engineered cells tolerate large amounts of branched-chain fatty acid in the growth medium, plasma membrane, or lipid droplets, and/or produce branched-chain fatty acid more economically than an unmodified cell by, e.g., using a less expensive feedstock, requiring less fermentation time, and the like.

[0202] The polynucleotide(s) encoding one or more polypeptides that catalyze the reaction(s) for producing branched-chain fatty acid may be derived from any source. Depending on the embodiment of the invention, the polynucleotide is isolated from a natural source such as bacteria, algae, fungi, plants, or animals; produced via a semi-synthetic route (e.g., the nucleic acid sequence of a polynucleotide is codon-optimized for expression in a particular host cell, such as *E. coli*); or synthesized de novo. In certain embodiments, it is advantageous to select an enzyme from a particular source based on, e.g., the substrate specificity of the enzyme, the type of branched-chain fatty acid produced by the source, or the level of enzyme activity in a given host cell. In one aspect of the invention, the enzyme and corresponding polynucleotide are naturally found in the host cell and overexpression of the polynucleotide is desired. In this regard, in some instances, additional copies of the polynucleotide are introduced in the host cell to increase the amount of enzyme available for fatty acid production. Overexpression of a native polynucleotide also is achieved by upregulating endogenous promoter activity, or operably linking the polynucleotide to a more robust promoter. Exogenous enzymes and their corresponding polynucleotides also are suitable for use in the context of the invention, and the features of the biosynthesis pathway or end product can be tailored depending on the particular enzyme used. If desired, the polynucleotide(s) is isolated or derived from the branched-chain fatty acid-producing organisms described herein.

[0203] In certain embodiments, the cell produces an analog or variant of a polypeptide described herein. Amino acid sequence variants of the polypeptide include substitution, insertion, or deletion variants, and variants may be substantially homologous or substantially identical to the unmodified polypeptides as set out above. In certain embodiments, the variants retain at least some of the biological activity, e.g., catalytic activity, of the polypeptide. Other variants include variants of the polypeptide that retain at least about 50%, preferably at least about 75%, more preferably at least about 90%, of the biological activity.

[0204] Substitution variants typically exchange one amino acid for another at one or more sites within the protein. Substitutions of this kind can be conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine.

[0205] In some instances, the recombinant cell comprises an analog or variant of the exogenous or overexpressed polynucleotide(s) described herein. Nucleic acid sequence variants include one or more substitutions, insertions, or deletions, and variants may be substantially homologous or substantially identical to the unmodified polynucleotide. Polynucleotide variants or analogs encode mutant enzymes having at least partial activity of the unmodified enzyme. Alternatively, polynucleotide variants or analogs encode the same amino acid sequence as the unmodified polynucleotide. Codon-optimized sequences, for example, generally encode the same amino acid sequence as the parent/native sequence but contain codons that are preferentially expressed in a particular host organism.

[0206] A polypeptide or polynucleotide "derived from" an organism contains one or more modifications to the native amino acid sequence or nucleotide sequence and exhibits similar, if not better, activity compared to the native enzyme (e.g., at least 70%, at least 80%, at least 90%, at least 95%, at least 100%, or at least 110% the level of activity of the native enzyme). For example, enzyme activity is improved in some contexts by directed evolution of a parent/native sequence. Additionally or alternatively, an enzyme coding sequence is mutated to achieve feedback resistance. Thus, in one or more embodiments of the invention, the polypeptide encoded by the exogenous polynucleotide is feedback resistant and/or is modified to alter the activity of the native enzyme. A polynucleotide "derived from" a reference polynucleotide encompasses, but is not limited to, a polynucleotide comprising a nucleic acid sequence that has been codon-optimized for expression in a desired host cell.

[0207] The cell of the invention may comprise any combination of polynucleotides described herein to produce branched-chain fatty acid comprising a methyl branch on one or more even number carbons. For example, the invention provides a cell comprising (i) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding an acyl transferase lacking polyketide synthesis activity, and (ii) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a propionyl-CoA carboxylase and/or an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA mutase, wherein the polynucleotide(s) are expressed and the cell produces more branched-chain fatty acid comprising a methyl on one or more even number carbons than an otherwise similar cell that does not comprise the polynucleotide(s). Recombinant cells can be produced in any suitable manner to establish an expression vector within the cell. The expression vector can include the exogenous polynucleotide operably linked to expression elements, such as, for example, promoters, enhancers, ribosome binding sites, operators and activating sequences. Such expression elements may be regulatable, for example, inducible (via the addition of an inducer). Alternatively or in addition, the expression vector can include additional copies of a polynucleotide encoding a native gene product operably linked to expression elements. Representative examples of useful promoters include, but are not limited to: the LTR (long terminal 35 repeat from a retrovirus) or SV40 promoter, the *E. coli* lac,

tet, or trp promoter, the phage Lambda P_L promoter, and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. In one aspect, the expression vector also includes appropriate sequences for amplifying expression. The expression vector can comprise elements to facilitate incorporation of polynucleotides into the cellular genome. Introduction of the expression vector or other polynucleotides into cells can be performed using any suitable method, such as, for example, transformation, electroporation, microinjection, microprojectile bombardment, calcium phosphate precipitation, modified calcium phosphate precipitation, cationic lipid treatment, photoporation, fusion methodologies, receptor mediated transfer, or polybrene precipitation. Alternatively, the expression vector or other polynucleotides can be introduced by infection with a viral vector, by conjugation, by transduction, or by other any other suitable method.

[0208] Cells, such as bacterial cells, containing the polynucleotides encoding the proteins described herein can be cultured under conditions appropriate for growth of the cells and expression of the polynucleotides. Cells expressing the protein can be identified by any suitable methods, such as, for example, by PCR screening, screening by Southern blot analysis, or screening for the expression of the protein. In certain embodiments, cells that contain the polynucleotide(s) can be selected by including a selectable marker in the DNA construct, with subsequent culturing of cells containing a selectable marker gene, under conditions appropriate for survival of only those cells that express the selectable marker gene. The introduced DNA construct can be further amplified by culturing genetically modified cells under appropriate conditions (e.g., culturing genetically modified cells containing an amplifiable marker gene in the presence of a concentration of a drug at which only cells containing multiple copies of the amplifiable marker gene can survive). Cells that contain and express polynucleotides encoding the exogenous proteins can be referred to herein as genetically modified cells. Bacterial cells that contain and express polynucleotides encoding the exogenous protein can be referred to as genetically modified bacterial cells.

[0209] Exemplary cells of the invention include *E. coli* BW25113 comprising pTrcHisA mmat and pZA31-accA1-pccB, which was deposited with American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Va., on Dec. 14, 2010, under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure ("Budapest Treaty"), and assigned Deposit Accession No. [XXX] on [DATE], and *E. coli* BL21 Star (DE3) comprising pTrcHisA Ec sbm So ce epi and pZA31 mmat which was deposited with American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Va., on Dec. 14, 2010, under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure ("Budapest Treaty"), and assigned Deposit Accession No. [XXX] on [DATE]. The invention also includes variants or progeny of the cells described herein that retain the phenotypic characteristics of the recombinant microbe. A substantially pure monoculture of the cell described herein (i.e., a culture comprising at least 80% or at least 90% of a desired cell) also is provided.

[0210] Any cell culture conditions appropriate for growing a host cell and synthesizing branched-chain fatty acid is suitable for use in the inventive method. Addition of fatty acid synthesis intermediates, precursors, and/or co-factors for the enzymes associated with branched-chain fatty acid synthesis to the culture is contemplated herein. In certain embodiments, the genetically modified cells (such as genetically modified bacterial cells) have an optimal temperature for growth, such as, for example, a lower temperature than normally encountered for growth and/or fermentation. For example, in certain embodiments, incorporation of branched-chain fatty acids into the membrane may increase membrane fluidity, a property normally associated with low growth temperatures. In addition, in certain embodiments, cells of the invention may exhibit a decline in growth at higher temperatures as compared to normal growth and/or fermentation temperatures as typically found in cells of the type.

[0211] The inventive method optionally comprises extracting branched-chain fatty acid from the culture. Fatty acids can be extracted from the culture medium and measured using any suitable manner. Suitable extraction methods include, for example, methods as described in: Bligh et al., A rapid method for total lipid extraction and purification, *Can. J. Biochem. Physiol.* 37:911-917 (1959). In certain embodiments, production of fatty acids in the culture supernatant or in the membrane fraction of recombinant cells can be measured. In this embodiment, cultures are prepared in the standard manner, although nutrients (e.g., 2-methylbutyrate, isoleucine) that may provide a boost in substrate supply can be added to the culture. Cells are harvested by centrifugation, acidified with hydrochloric or perchloric acid, and extracted with chloroform and methanol, with the fatty acids entering the organic layer. The fatty acids are converted to methyl esters, using methanol at 100° C. The methyl esters are separated by gas chromatography (GC) and compared with known standards of fatty acids (purchased from Larodan or Sigma). Confirmation of chemical identity is carried out by combined GC/mass spec, with further mass spec analysis of fragmented material carried out if necessary.

[0212] In one embodiment, the cell utilizes the branched-chain fatty acid as a precursor to make one or more other products. Products biosynthesized (i.e., derived) from branched-chain fatty acid include, but are not limited to, phospholipids, triglycerides, alkanes, olefins, wax esters, fatty alcohols, and fatty aldehydes. Some host cells naturally generate one or more products derived from branched-chain fatty acid; other host cells are genetically engineered to convert branched-chain fatty acid to, e.g., an alkane, olefin, wax ester, fatty alcohol, phospholipid, triglyceride, and/or fatty aldehyde. Organisms and genetic modifications thereof to synthesize products derived from branched-chain fatty acids are further described in, e.g., International Patent Publication Nos. WO 2007/136762, WO 2008/151149, and WO 2010/062480, and U.S. Patent Application Publication US 2010/0298612, all of which are hereby incorporated by reference in their entirety. In one aspect, the inventive method comprises extracting a product derived from branched-chain fatty acid (phospholipid, triglyceride, alkane, olefin, wax ester, fatty alcohol, and/or fatty aldehyde synthesized in the cell from branched-chain fatty acid) from the culture. Any extraction method is appropriate, including the extraction methods described in International Patent Publication Nos. WO 2007/136762, WO 2008/151149, and WO 2010/062480, and U.S. Patent Application Publication Nos. US 2010/0251601, US 20100242345, US 20100105963, and US 2010/0298612.

[0213] The inventive cell preferably produces more branched-chain fatty acid comprising a methyl branch on one

or more even number carbons than an otherwise similar cell that does not comprise the polynucleotide(s). Methods of measuring fatty acid released into the fermentation broth or culture media or liberated from cellular fractions are described herein. Branched-chain fatty acid production is not limited to fatty acid accumulated in the culture, however, but also includes fatty acid used as a precursor for downstream reactions yielding products derived from branched-chain fatty acid. Thus, products derived from branched-chain fatty acid (e.g., phospholipids, triglycerides, fatty alcohols, olefins, wax esters, fatty aldehydes, and alkanes) are, in some embodiments, surrogates for measuring branched-chain fatty acid production in a host cell. Methods of measuring fatty acid content in phospholipid in the cell membrane are described herein. Similarly, measurement of degradation products of branched-chain fatty acids also is instructive as to the amount of branched-chain fatty acid is produced in a host cell. Depending on the particular embodiment of the invention, the inventive cell produces at least 3%, at least 5%, at least 10%, at least 20%, at least 25%, or at least 50% more branched-chain fatty acid than an otherwise similar cell that does not comprise the polynucleotide(s).

[0214] The invention further provides a composition comprising the branched-chain fatty acids described herein. For example, the invention provides a composition comprising a branched-chain fatty acid comprising between 10-18 carbons in the carbon backbone, such as fatty acids comprising between 10 and 16 carbons (e.g., fatty acids comprising 10, 11, 12, 13, 14, 15, or 16 carbons), with branching on one or more even numbered carbons (e.g., C2, C4, C6, C8, C10, C12, C14, and/or C16). A composition comprising longer-chain fatty acid also is provided, such as a composition comprising between 19 and 22 carbons in the longest carbon chain. A composition comprising a combination of any of the fatty acids described herein also is provided (e.g., a composition comprising fatty acids of varying lengths and/or branch locations along the carbon backbone).

[0215] The following examples further describe and demonstrate embodiments within the scope of the invention. The examples are given solely for the purpose of illustration and are not to be construed as limitations of the invention, as many variations thereof are possible without departing from the spirit and scope of the invention.

Example 1

Construction of Methylmalonyl-CoA Mutase Expression Vector

[0216] There are numerous genes annotated to encode the two subunits of methylmalonyl-CoA mutase. *Janibacter* sp. HTCC2649 encodes two such genes. Synthetic versions of these genes were prepared, with the codon usage altered to match that used by many *E. coli* genes (i.e., the coding sequence was codon-optimized for expression in *E. coli*). By analogy to other methylmalonyl-CoA mutase genes, these synthetic genes were named mutA (SEQ ID NO: 1) and mutB (SEQ ID NO: 2), corresponding to the MutA (SEQ ID NO: 3) and MutB (SEQ ID NO: 4) protein subunits. In the synthetic DNA, an extra three base pairs were added (encoding an alanine residue immediately after the initiation methionine) in mutA to facilitate introduction of an NcoI site. An XhoI restriction site was also placed after the coding sequence of mutB for insertion into the pBAD vector (Invitrogen). The NcoI/XhoI fragment was cloned into pBAD.

Example 2

Construction of Methylmalonyl-CoA Epimerase Expression Vector

[0217] There are numerous genes annotated to encode methylmalonyl-CoA mutase. One such gene is from *Streptomyces sviceus*. A synthetic gene can be constructed (SEQ ID NO: 5) using codon usage similar to *E. coli* genes and with EcoRI and Hind III sites flanking the coding region. An *E. coli* Shine-Dalgarno sequence can be added between the EcoRI site and the initiation codon for the epimerase gene. The predicted protein product is the same as the predicted protein product from the *S. sviceus* gene (SEQ ID NO: 6). The epimerase gene can be cloned into the pBAD-mutAB construct using the EcoRI and Hind III restriction sites (downstream of mutB) to form the pBAD-mutAB-epimerase gene plasmid. *E. coli* cultures can be grown at 27° C. after induction with arabinose and supplemented with hydroxycobalamin to achieve expression of functional methylmalonyl-CoA mutase and branched-chain fatty acid production.

Example 3

Construction of Propionyl-CoA Carboxylase Expression Vector

[0218] Nucleotide sequences (SEQ ID NO: 7 and SEQ ID NO: 8) encoding the two propionyl-CoA carboxylase subunits AccA1 (GenBank Accession NO. AF113603.1; SEQ ID NO: 9) and PccB (GenBank Accession No. AF113605.1; SEQ ID NO: 10)), respectively, from the *Streptomyces coelicolor* A3(2) propionyl-CoA carboxylase (Rodriguez E., Gramajo H., *Microbiology.* 1999 November; 145:3109-19), were codon-optimized for *E. coli* expression. A gene construct for expressing propionyl-CoA carboxylase was constructed with the following elements sequentially 1) $P_{LlacO\text{-}1}$ promoter and operator plus T7 gene10 ribosomal binding site (SEQ ID NO: 11); 2) optimized accA1 (SEQ ID NO: 12); 3) three restriction site sequences including BglII, NotI and XbaI and a T7 gene10 ribosome binding site (SEQ ID NO: 13); and 4) codon-optimized pccB (SEQ ID NO: 14). The synthesized DNA fragments were cloned into the XhoI and PstI sites of expression vector pZA31-MCS (Expressys, Ruelzheim, Germany), resulting in plasmid pZA31-accA1-pccB (SEQ ID NO: 15).

Example 4

Construction of Propionyl-CoA Synthetase Expression Vector

[0219] The *Salmonella enterica* propionyl-CoA synthetase gene, prpE, was amplified using PCR and the primers set forth in SEQ ID NO: 16 and SEQ ID NO: 17, and placed behind a Shine-Dalgarno sequence in the plasmid pZA31-accA1-pccB (SEQ ID NO: 15) using the restriction enzymes PstI and BamHI. Enhanced propionyl-CoA synthetase production is expected to increase synthetic flux to propionyl-CoA.

Example 5

Reduction of Propionylation of Propionyl-CoA Synthetase

[0220] In *S. enterica*, propionyl-CoA synthetase is subject to inhibition by propionylation at lysine 592 when propionyl-CoA levels accumulate. (Garrity et al, *J. Biol. Chem., Vol.*

282, Issue 41, 30239-30245, Oct. 12, 2007). Similar enzyme modulation may occur in other species, although the position of the modified lysine may be different. Several strategies to overcome this inhibition will be tested and compared. First, the propionyl-CoA synthetase gene will be mutated to change the coding capacity from lysine (at the site of propionylation) to arginine or other amino acids to prevent propionylation. Second, a source of resveratrol or other sirtuin activators will be introduced into the culture medium to activate sirtuin to depropionylate PrpE. Third, the endogenous N-acetyltransferase enzyme responsible for the propionylation reaction will be knocked out. For example, if working with *S. enterica*, pat could be deleted. As another example, if working with *B. subtilis*, acuA could be deleted. Fourth, the flux of propionyl-CoA into fatty acid synthesis will be increased by increasing propionyl-CoA carboxylase activity to keep free propionyl-CoA levels down. Fifth, the sirtuin activity will be increased, thus increasing deacetylation of propionyl-CoA carboxylase. For example, the *S. enterica* cobB expression could be increased.

Example 6

Creation of an Expression Vector Comprising the Coding Sequence of the MMAT (Methylmalonyl-CoA Acyl Transferase) Domain from *Mycobacterium* Mycocerosic Acid Synthase (MAS).

[0221] *Mycobacterium* MAS is a multifunctional protein that catalyzes the synthesis of mycocerosic acid and that contains a domain with MMAT activity. The MMAT domain (amino acids 508-890) (SEQ ID NO: 18) of MAS from *Mycobacterium bovis* BCG (YP_979046) (SEQ ID NO: 19) was codon optimized for *E. coli* expression (SEQ ID NO: 20). The optimized sequence was synthesized and cloned into vector pTrcHisA (Invitrogen) between the BamHI and HindIII sites. The resulting construct fused the MMAT domain with the His tag leader peptide encoded by the vector. The expression vector was introduced into a recombinant *E. coli* host that produces methylmalonyl-CoA. MMAT activity catalyzes the formation of methylmalonyl-ACP, which subsequently can be incorporated into the type II fatty acid synthesis pathway to form methyl branches at even positions of the fatty acid chain.

Example 7

Method for Detecting Acyl-CoA

[0222] This example describes an exemplary method for detecting and quantifying an acyl-CoA (e.g., methylmalonyl-CoA) in a sample, such as a sample of recombinant host cells producing branched-chain fatty acid.

[0223] A stable, labeled (deuterium) internal standard-containing master mix was prepared comprising d_3-3-hydroxymethylglutaryl-CoA (200 μl of 50 μg/ml stock in 10 ml of 15% trichloroacetic acid). An aliquot (500 μl) of the master mix was added to a 2 ml tube. Silicone oil (AR200; Sigma catalog number 85419; 800 μl) was layered onto the master mix. An *E. coli* culture (800 μl) was layered gently on top of the silicone oil, and the resulting sample was subjected to centrifugation at 20,000×g for five minutes at 4° C. in an Eppendorf 5417 C centrifuge. A portion (300 μl) of the master mix-containing layer was transferred to an empty tube and frozen on dry ice for 30 minutes.

[0224] The acyl-CoA content of samples was determined using HPLC/MS/MS. Individual coenzyme-A standards (propionyl-CoA, methylmalonyl-CoA, succinyl-CoA, malonyl-CoA, isobutyryl-CoA, isovaleryl-CoA, and acetyl-CoA) were purchased from Sigma Chemical Company (St. Louis, Mo.) and prepared as 500 μg/ml stocks in methanol. The analytes were pooled, and standards with all of the analytes were prepared by dilution with 15% trichloroacetic acid. Standards for regression were prepared by transferring 500 μl of the working standards to an autosampler vial containing 10 μL of the 50 μg/ml internal standard. Sample peak areas (or heights) were normalized to the stable-labeled internal standard (d_3-3-hydroxymethylglutaryl-CoA, Cayman Chemical Co.). Samples were assayed by HPLC/MS/MS on a Sciex API5000 mass spectrometer in positive ion Turbo Ion Spray. Separation was carried out by reversed-phase high performance liquid chromatography using a Phenomenex Onyx Monolithic C18 column (2×50 mm) and mobile phases of (1) 5 mM ammonium acetate, 5 mM dimethylbutylamine, 6.5 mM acetic acid and (2) acetonitrile with 0.1% formic acid, with the gradient set forth in Table C.

TABLE C

Time	Mobile Phase A (%)	Mobile Phase B (%)
0 min	97.5	2.5
1.0 min	97.5	2.5
2.5 min	91.0	9.0
5.5 min	45	55
6.0 min	45	55
6.1 min	97.5	2.5
7.5 min	—	—
9.5 min	End Run	

[0225] The conditions on the mass spectrometer were: DP 160, CUR 30, GS1 65, GS2 65, IS 4500, CAD 7, TEMP 650 C. The transitions set forth in Table D were used for the multiple reaction monitoring (MRM).

TABLE D

Compound	Precursor Ion*	Product Ion*	Collision Energy	CXP
n-Propionyl-CoA	824.3	317.2	41	32
Methylmalonyl-CoA	868.1	317.1	42	31
Succinyl-CoA	868.2	361.1	49	38
Malonyl-CoA	854.2	347.2	41	36
Isobutyryl-CoA	838.3	345.2	45	34
Isovaleryl-CoA	852.2	345.2	45	34
Acetyl-CoA	810.3	303.2	43	30
d3-3-Hydroxymethylglutaryl-CoA	915.2	408.2	49	13

*Energy (Volts) for MS/MS analysis

Example 8

Analysis of Fatty Acids Produced by Host Cells

[0226] This example illustrates a method of analyzing branched-chain fatty acids produced by cells (e.g., recombinant microbes).

[0227] Cell cultures (approximately 1.5 ml) were frozen in 2.0 ml glass vials and stored at −20° C. until ready for processing. Samples were chilled on dry ice for 30 minutes and lyophilized overnight (−16 hours) until dry. A 10 μl aliquot of internal standard (glyceryl trinonadecanoate (Sigma catalog number T4632-1G)) was added to each vial, followed by 400

μL of 0.5 N NaOH (in methanol). The vial was capped and vortexed for 10 seconds. Samples were incubated at 65°C. for 30-50 minutes. Samples were then removed from the incubator, and 500 μl of boron trifluoride reagent (Aldrich catalog number B1252) was added. The samples were vortexed again for 10 seconds, incubated at 65° C. for 10-15 minutes, and cooled to room temperature (approximately 20 minutes). Hexane (350 μl) was added, and the samples were again vortexed for 10 seconds. If the phases did not separate, 50-100 μl of saturated salt solution (5 g NaCl to 5 ml water) was added, and the sample was vortexed for 10 seconds. At least 100 μl of the top hexane layer was placed into the gas chromatography vial. The vial was capped and stored at 4° C. until analyzed by gas chromatography.

[0228] Gas chromatography was performed as described in Table E below. A bacterial acid methyl ester standard (Sigma catalog number 47080-U) and a fatty acid methyl ester standard (Sigma catalog number 47885-U) were used to identify peaks in samples. A sample check standard using glyceryl tripalmitate (Sigma catalog number T5888-1G) was used to confirm esterification of samples. A blank standard (internal standard only) was used to assess background noise.

TABLE E

Gas Chromatograph	HP 5890 GC Series II
Detector	FID 360° C. 40 ml/min Hydrogen, 400 ml/min Air
Carrier Gas	Helium
Quantitative Program	GC Chemstation A.09.03. (Agilent)
Column	VF-5 ms 15 M × 0.150 mm × 0.15 μm Varian catalog number CP9035
Injection Liner	Gooseneck (with glass wool packing)
Injector	HP 7673
Injection Syringe	10 μL
Injection Mode	Split 25:1
Injection volume	4 μL (Plunger Speed = fast; 5 sample pumps)
Pre Injection Solvent Washes	2 samples
Post Injection Solvent Washes	3 for both acetone and hexane
Injector Temperature	325° C.
Total Program Time	16 minutes

	Initial Temp. (° C.)	Initial Time (min)	Rate (° C./min)	Final Temp (° C.)	Final Time (min)
Thermal Program	90	0.75	20.0	325	1.0
			25.0	350	2.5

Example 9

Construction of Expression Vectors Comprising *S. Cinnamonensis* mutA and mutB and *S. sviceus* epi.

[0229] A synthetic DNA construct was generated comprising *Streptomyces cinnamonensis* mutA (SEQ ID NO: 24) (GenBank Accession No. AAA03040.1), *S. cinnamonensis* mutB (SEQ ID NO: 25) (GenBank Accession No. AAA03041.1), and a *Streptomyces sviceus* ATCC 29083 methylmalonyl-CoA epimerase gene (SEQ ID NO: 26) (GenBank Accession No. ZP_06919825.1). The genes were codon-optimized for expression in *E. coli*. An EcoRI restriction site was placed on the 5' end, and a BamHI site was placed on the 3' end of the synthesized gene construct. These sites were subsequently used for cloning into a pZA31 vector (Expressys, Ruelzheim, Germany). A ribosome binding sequence and spacer was placed before the mutA and epimerase gene start codons (SEQ ID NO: 27). The plasmid was designated pZA31 mutAB Ss epi.

Example 10

Construction of Expression Vectors Comprising Sbm and malE/sbm Polynucleotides

[0230] Sleeping beauty mutase (Sbm) (also known as methylmalonyl-CoA mutase (MCM)) is an enzyme that catalyzes the rearrangement of succinyl-CoA to L-methylmalonyl-CoA. The enzyme is vitamin B12 (cobalamin) dependent. Methylmalonyl-CoA is a building block for scattered branch-chain fatty acids (sBCFA) (i.e., branched-chain fatty acid comprising a methyl branch on one or more even number carbons of the fatty acid backbone). Plasmids comprising a polynucleotide encoding Sbm were generated to introduce multiple copies of the Sbm coding sequence, downstream of a regulatable promoter, into *E. coli* host cells.

[0231] A polynucleotide was synthesized based on the sequence of *E. coli* sbm (SEQ ID NO: 28) (GenBank Accession No. NP_417392.1) from *E. coli* strain MG1655. The nucleic acid sequence was codon-optimized to match the pattern of highly expressed *E. coli* genes while maintaining the native amino acid sequence of the enzyme. The generated nucleic acid sequence is set forth in SEQ ID NO: 29. A BamHI and an XbaI site were added at the 5' end of the synthetic Sbm coding sequence with the sequence GGATCCATGTCTAGA (SEQ ID NO: 49) adjacent to the ATG translation initiation sequence. A SacI restriction site sequence was added to the 3' end of the synthetic Sbm coding sequence. The gene was synthesized, cloned into a pUC57 vector, and sequenced (GenScript, Piscataway, N.J.). The synthetic sbm was then released from pUC57 by restriction enzymes BamHI and Sad, and sub-cloned into plasmid pTrcHisA (Invitrogen, Carlsbad, Calif.) in frame with the poly-histidine sequence (GenScript, Piscataway, N.J.). The plasmid was designated pTrcHisA Ec sbm. The sequence was confirmed by sequencing (GenScript, Piscataway, N.J.). The recombinant protein encoded by the sequence contained a poly-histidine sequence (Met-Gly-Gly-Ser-His-His-His-His-His-His-Gly-Met-Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly-Arg-Thr-Asp-Asp-Asp-Asp-Lys-Asp-Arg-Trp-Gly-Ser (SEQ ID NO: 50)) and a full-length native Sbm amino acid sequence.

[0232] A recombinant methylmalonyl-CoA mutase has been reported to be insoluble in *E. coli* (Korotkova, N., and M. E. Lidstrom. *J. Biological Chemistry* 279: 13652-8 (2004)). Translation fusion with maltose-binding protein (MBP, encoded by malE) prevents aggregation of recombinant proteins (Kapust, R. B., and D. S. Waugh. Protein Science 8: 1668-74 (1999)). A recombinant construct was generated by inserting malE upstream of sbm. The malE polynucleotide was synthesized based on the sequence of maltose binding protein (*E. coli* MG1655 GenBank NC_000913.2 (GenScript, Piscataway, N.J.)). A BamHI site was placed adjacent to the translation initiation codon of malE, and an XbaI site was placed immediately 5' to the stop codon of the malE sequence (SEQ ID NO: 30). Also, one nucleotide was changed (T438 to C438) to remove a restriction site recognition sequence for BglII.

[0233] The MalE coding sequence (SEQ ID NO: 30) was first synthesized and cloned into a pUC57 plasmid. After confirming its sequence, the malE polynucleotide was released using restriction enzymes BamHI and XbaI. The

released malE was then re-cloned into plasmid pTrcHisA Ec sbm at BamHI and XbaI sites (GenScript, Piscataway, N.J.). The resulting plasmid was designated pTrcHisA Ec malE Ec sbm. The recombinant protein encoded by pTrcHisA Ec malE Ec sbm contains three peptides: the poly-histidine tag, full-length MBP, and full-length Sbm.

Example 11

Construction of a Recombinant Expression Vector Comprising a Polynucleotide Encoding the Methylmalonyl-CoA Acyl Transferase (MMAT) Domain from *Mycobacterium* Mycocerosic Acid Synthase (MAS).

[0234] *Mycobacterium* MAS is a multifunctional protein containing MMAT activity that catalyzes the synthesis of mycocerosic acid. The nucleic acid sequence encoding the MMAT domain (amino acids 508-890) (SEQ ID NO: 18) of MAS from *Mycobacterium bovis* BCG (GenBank Accession No. YP_979046) (SEQ ID NO: 19) was codon-optimized for *E. coli* expression (SEQ ID NO: 20). The optimized sequence, designated "mmat," was synthesized and cloned into vector pTrcHisA (Invitrogen) between the BamHI and HindIII sites. The resulting construct fused the MMAT domain with the poly-histidine tag encoded by the vector. The expression vector (pTrcHisA mmat) was introduced into a recombinant *E. coli* host that produces methylmalonyl-CoA. MMAT activity catalyzes the formation of methylmalonyl-ACP, which is incorporated by Type II fatty acid synthase into fatty acid, forming methyl branches at even positions of the fatty acid chain.

[0235] An expression vector encoding *Mycobacterium bovis* BCG fused to a poly-histidine tag also was generated. The pTrcHisA mmat plasmid DNA described above was amplified by PCR using oligonucleotides synthesized to include 5'-KpnI (SEQ ID NO: 31) and 3'-HindIII restriction sites (SEQ ID NO: 32) (Integrated DNA Technologies, Inc., Coralville, Iowa). PCR was run on samples having 1 μl (2 ng) pTrcHisA mmat DNA, 1.5 μl of a 10 μM stock of each primer, 5 μl of 10× Pfx reaction mix (Invitrogen Carlsbad, Calif.), 0.5 μl of Pfx DNA polymerase (1.25 units), and 41 μl of water. PCR conditions were as follows: the samples were initially incubated at 95° C. for three minutes, followed by 30 cycles at 95° C. for 30 seconds (strand separation), 58° C. for 30 seconds (primer annealing), and 68° C. primer extension for 1.5 minutes. Following the cycles, the samples were incubated for 10 minutes at 68° C., and the samples were then held at 4° C.

[0236] The PCR products were purified using a QIAquick® PCR Purification Kit (Qiagen), digested with restriction enzymes KpnI and HindIII and ligated (Fast-Link Epicentre Biotechnologies, Madison, Wis.) with KpnI/HindIII-digested pZA31MCS (Expressys, Ruelzheim, Germany). The ligation mix was used to transform *E. coli* DHSα™ (Invitrogen Carlsbad, Calif.). Isolated colonies were screened by PCR using a sterile pipette tip stab as an inoculum into a reaction tube containing only water, followed by addition of the remaining PCR reaction cocktail (AccuPrime™ SuperMixII, Invitrogen Carlsbad, Calif.) and primers as described above.

[0237] Recombinant plasmids were isolated and purified using the QIAPrep® Spin Miniprep Kit (Qiagen) and characterized by restriction enzyme digestion (DraI, KpnI and HindIII from New England Biolabs, Beverly, Mass.). The plasmids were subsequently used to transform BW25113 (*E. coli* Genetics Stock Center, New Haven, Conn.) made competent using the calcium chloride method. Transformants were selected on Luria agar plates containing 34 μg/ml chloramphenicol. Plasmid DNA was isolated and purified using the QIAfilter™ Plasmid Midi Kit (Qiagen). DNA sequencing confirmed that the insert was mmat (SEQ ID NO: 34). The resulting plasmid incorporating a poly-histidine tag was designated pZA31 mmat.

Example 12

Method of Generating a Recombinant Host Cell Comprising an Exogenous Polynucleotide Encoding a Propionyl-CoA Carboxylase and an Exogenous Polynucleotide Encoding a Methylmalonyl-CoA Acyl Transferase (MMAT) Domain from *Mycobacterium* Mycocerosic Acid Synthase (MAS).

[0238] This example describes an exemplary method for making a cell comprising an exogenous polynucleotide comprising a nucleic acid sequence encoding a polypeptide that catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA and an exogenous polynucleotide comprising a nucleic acid sequence encoding a polypeptide that catalyzes the conversion of methylmalonyl-CoA to methylmalonyl-ACP. The method entails co-transduction of *E. coli* with plasmids containing a propionyl-CoA carboxylase gene from *Streptomyces coelicolor* and a gene encoding a MMAT domain from *Mycobacterium* MAS.

[0239] *E. coli* BW25113 cells (*E. coli* Genetic Stock Center, New Haven, Conn.) were made chemically competent for plasmid DNA transformation by a calcium chloride method. Actively growing 50 ml *E. coli* cultures were grown to an optical density (at 600 nm) of ~0.4. Cultures were quickly chilled on ice, and the bacteria were recovered by centrifugation at 2700×g for 10 minutes. The supernatant was discarded and pellets were gently suspended in 30 ml of an ice-cold 80 mM $MgCl_2$, 20 mM $CaCl_2$ solution. Cells were again recovered by centrifugation at 2700×g for 10 minutes. The supernatant was discarded and pellets were gently resuspended in 2 ml of an ice-cold 0.1 M $CaCl_2$ solution.

[0240] Cells were transformed on ice in pre-chilled 14 ml round-bottom centrifuge tubes. Approximately 25 ng of each of pTrcHisA mmat and pZA31-accA1-pccB (described above) was incubated on ice with 100 μl of competent cells for 30 minutes. The cells were heat shocked at 42° C. for 90 seconds and immediately placed on ice for two minutes. Pre-warmed SOC medium (500 μl; Invitrogen, Carlsbad, Calif.) was added and the cells allowed to recover at 37° C. with 225 rpm shaking. A portion (50 μl) of the transformed cell mix was spread onto selective LB agar 100 mg/ml ampicillin and 34 mg/ml chloramphenicol plates to select for cells carrying the pTrcHisA mmat and pZA31/32-accA1-pccB plasmids. Individual colonies were picked from each plate and streaked onto LB agar (with ampicillin and chloramphenicol) to confirm the antibiotic resistance phenotype. Restriction endonuclease digestion analysis of isolated plasmid DNA with HaeII verified the plasmid DNA pool for each strain. A sample of *E. coli* BW25113 comprising pTrcHisA mmat and pZA31-accA1-pccB was deposited with American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Va., on Dec. 14, 2010, under the provisions of the Budapest Treaty for the International Recognition of the

Deposit of Microorganisms for the Purpose of Patent Procedure ("Budapest Treaty"), and assigned Deposit Accession No. [XXX] on [DATE].

Example 13

Construction of an Expression Vector Encoding *Sorangium Cellulosum* So ce 56 Methylmalonyl-CoA Epimerase

[0241] A *S. cellulosum* methylmalonyl-CoA epimerase synthetic gene (So ce epi) was designed and synthesized (SEQ ID NO: 37). The coding sequence was codon-optimization for expression in *E. coli* and modified to remove restriction sites (GenScript, Piscataway, N.J.). The nucleic acid sequence was flanked with a SacI site and a synthetic ribosome binding site from the pBAD vector (Invitrogen, Carlsbad, Calif.) adjacent to the translation initiation sequence (SEQ ID NO: 39). The synthetic gene was cloned as a SacI/PstI fragment into pTrcHisA Ec sbm and pTrcHisA Ec malE Ec sbm, with the resulting plasmids designated as pTrcHisA Ec sbm So ce epi and pTrcHisA Ec malE Ec sbm So ce epi, respectively.

Example 14

Construction of an Expression Vector Encoding *Kribbella Flavida* DSM 17836 Methylmalonyl-CoA Epimerase

[0242] A *K. flavida* methylmalonyl-CoA epimerase gene (Kf epi) was designed and synthesized (SEQ ID NO: 35). The coding sequence was optimized for expression in *E. coli* and restriction sites were removed (GenScript, Piscataway, N.J.). The gene was flanked with a Sad site and a synthetic ribosome binding site from the pBAD vector adjacent to the translation initiation sequence (SEQ ID NO: 39). The synthetic gene was cloned as a SacI/PstI fragment into pTrcHisA Ec sbm and pTrcHisA Ec malE Ec sbm. The resulting plasmids were designated pTrcHisA Ec sbm Kf epi and pTrcHisA Ec malE Ec sbm Kf epi, respectively.

Example 15

Production of Host Cells Producing Branched-Chain Fatty Acid

[0243] This example describes the production of branched-chain fatty acid using a recombinant host cell (e.g., *E. coli*) expressing polynucleotides encoding a propionyl-CoA carboxylase or a methylmalonyl-CoA mutase and a methylmalonyl-CoA epimerase, in some instances in conjunction with a polynucleotide encoding an acyl transferase and/or thioesterase.

[0244] It is useful to have the capacity to tailor the fatty acid chain length. Branched fatty acids of different lengths have different physical properties suitable for different commercial applications. To demonstrate the capacity to tailor the chain length of branched fatty acids, *E. coli* 'TesA (Cho, H., and J. E. Cronan, Jr. *J. Biological Chemistry* 270: 4216-9 (1995)) was incorporated into expression vectors described above and inserted into host cells. To create a pTrc Ec 'tesA expression vector, a truncated *E. coli* tesA ('tesA) cDNA (SEQ ID NO: 40) was created by PCR amplification of the *E. coli* tesA gene (GenBank Accession No. L06182). A 5' primer (SEQ ID NO: 41) was designed to anneal after the 26th codon of tesA, modifying the 27th codon from an alanine to a methionine and creating a NcoI restriction site. A 3' primer (SEQ ID NO: 43) incorporating a BamHI restriction site was designed. PCR was performed with 50 μl of Pfu Ultra II Hotstart 2× master mix (Agilent Technologies, Santa Clara, Calif.), 1 μl of a mix of the two primers (10 μmoles of each), 1 μl of *E. coli* BW25113 genomic DNA, and 48 μl of water. PCR began with a two minute incubation at 95° C., followed by 30 cycles of 20 seconds at 95° C. for denaturation, 20 seconds for annealing at 58° C., and 15 seconds at 72° C. for extension. The sample was incubated at 72° C. for three minutes and then held at 4° C. The PCR product (Ec 'tesA) was purified using a QIAquick® PCR Purification Kit (Qiagen, Valencia, Calif.). The bacterial expression vector pTrcHisA and the 'tesA PCR product were digested with NcoI and BamHI. The digested vector and insert were ligated using Fast-Link (Epicentre Biotechnologies, Madison, Wis.). The ligation mix was then used to transform *E. coli* TOP 10 cells (Invitrogen, Carlsbad, Calif.). Recombinant plasmids were isolated using a QIAPrep0 Spin Miniprep Kit (Qiagen) and characterized by gel electrophoresis of restriction digests with HaeII. DNA sequencing confirmed that the 'tesA insert had been cloned and that the insert encoded the expected amino acid sequence (SEQ ID NO: 45). The resulting plasmid was designated pTrc Ec 'tesA.

[0245] To limit gene expression, the truncated *E. coli* 'tesA gene was subcloned into the low-copy bacterial expression vector pZS21-MCS (Expressys, Ruelzheim, Germany). The expression vector pTrc Ec 'tesA was a template in a PCR reaction using a 5' primer designed to create a flanking XhoI restriction site and include the pTrcHisA lac promoter (to replace the pZS21-MCS vector tet promoter) (SEQ ID NO: 46) and a 3' primer incorporating a HindIII restriction site (SEQ ID NO: 47). PCR was performed with 50 μl of Pfu Ultra II Hotstart 2× master mix (Agilent Technologies, Santa Clara, Calif.), 1 μl of a mix of the two primers (10 μmoles of each), 1 μl of pTrc Ec 'tesA plasmid DNA (6 ng), and 48 μl of water. PCR began with a two minute incubation at 95° C., followed by 30 cycles of 20 seconds at 95° C. for denaturation, 20 seconds for annealing at 57° C., and 20 seconds at 72° C. for extension. The sample was incubated at 72° C. for three minutes and then held at 4° C. The PCR product was purified using a QIAquick® PCR Purification Kit (Qiagen, Valencia, Calif.). The bacterial expression vector pZS21-MCS and the Ec 'tesA PCR product were digested with XhoI and HindIII. The digested vector and insert were ligated using Fast-Link (Epicentre Biotechnologies, Madison, Wis.). The ligation mix was then used to transform *E. coli* TOP10 cells (Invitrogen, Carlsbad, Calif.). Recombinant plasmids were isolated using a QIAPrep® Spin Miniprep Kit (Qiagen) and characterized by gel electrophoresis of restriction digests with HaeII. DNA sequencing confirmed that the 'tesA insert had been cloned and that the insert encoded the expected amino acid sequence (SEQ ID NO: 45). The resulting plasmid was designated pZS22 Ec 'tesA.

[0246] An *E. coli* strain deficient in fatty acid degradation (Voelker, T. A., and H. M. Davies. J. *Bacteriology* 176: 7320-7 (1994)) and able to regulate transcription of recombinant genes was generated as follows. An *E. coli* K-12 strain (K27) defective in fadD lacks the fatty acyl-CoA synthetase responsible for an initial step in fatty acid degradation. The strain K27 (F—, tyrT58(AS), fadD88, mel-1; CGSC Strain #5478) was obtained from the *E. coli* Genetic Stock Center (New Haven, Conn.). A genomic regulation cassette from strain DH5αZ1 [lacI^q, PN25-tetR, Sp^R, deoR, supE44, Δ(lac-

ZYA-argFV169), ϕ80 lacZΔM15 (Expressys, Ruelzheim, Germany)] was introduced into the host strain. The transducing phage P1vir was charged with DH5αZ1 DNA as follows. A logarithmically growing culture (5 ml LB broth containing 0.2% glucose and 5 mM $CaCl_2$) of donor strain, DH5αZ1, was infected with a 100 μl of a lysate stock of P1vir phage. The culture was further incubated three hours for the infected cells to lyse. The debris was pelleted, and the supernatant was further cleared through a 0.45 μm syringe filter unit. The fresh lysate was titered by spotting 10 μl of serial 1:10 dilutions of lysate in TM buffer (10 mM $MgSO_4$/10 mM Tris.Cl, pH 7.4) onto a 100 mm LB (with 2.5 mM $CaCl_2$) plate overlayed with a cultured lawn of *E. coli* in LB top agar (with 2.5 mM $CaCl_2$). The process was repeated using the newly created phage stock until the phage titer surpassed 10^9 pfu/ml.

[0247] The higher titer phage stock was used to transduce fragments of the DH5αZ1 genome into a recipient K27 strain. An overnight culture (1.5 ml) of K27 was pelleted and resuspended in 750 μl of a P1 salts solution (10 mM $CaCl_2$/5 mM $MgSO_4$). 100 μl of the suspended cells was inoculated with varying amounts of DH5αZ1 donor P1vir lysate (1, 10, and 100 μl) in sterile test tubes. The phage was allowed to adsorb to the cells for 30 minutes at 37° C. Absorption was terminated by addition of 1 ml LB broth plus 200 μl of 1 M sodium citrate, and the cultures were further incubated for 1 hour at 37° C. with aeration. The cultures were pelleted, and the cells suspended in 100 μl of LB broth (plus 0.2 M sodium citrate) and spread onto LB agar plates with 50 μg/mL spectinomycin. Spectinomycin-resistant strains were isolated, and genomic DNAs were screened by PCR for the presence of tetR, $lacI^q$ and fadD88. One such transductant was named K27-Z1 and used in further studies.

[0248] To transform K27-Z1, competent cells were placed on ice in pre-chilled 14 ml round bottom centrifuge tubes. Each plasmid was incubated with 50 μl of chemically competent K27-Z1 cells (Cohen, S. N., Change, A. C. Y., and L. Hsu. *Proceedings National Academy Sciences U.S.A.* 69: 2110-4 (1972)) for 30 minutes. The cells were heat shocked at 42° C. for 90 seconds and immediately placed on ice for two minutes. Pre-warmed SOC medium (250 μl) (Invitrogen, Carlsbad, Calif.) was added, and the cells were allowed to recover at 37° C. with 125 rpm shaking for one hour. Transformed cell mix (20 μl) was spread onto selective LB agar with 100 μg/ml ampicillin to select for cells carrying the pTrcHisA-based plasmids. Transformed cell mix (50 μl) was spread onto LB agar with 34 μg/ml chloramphenicol to select for cells carrying the pZA31-based plasmids. Transformed cell mix (150 μl) was spread onto LB agar with 100 μg/ml ampicillin and 34 μg/ml chloramphenicol to select for cells carrying both the pTrcHisA-based and pZA31-based plasmids. In some cases, the creation of triple transformants required two transformations: a double transformant was originally created, made competent, and transformed by a third plasmid.

[0249] Using the methods described above, *E. coli* strain K27-Z1 was transduced with pTrcHisA pZA31 (control), pZA31 mutAB Ss epi, pTrcHisA Ec sbm, and pTrcHisA Ec sbm/pZA31 Mb mmat. The bacteria were cultured in M9 with glycerol (0.2%) at 22° C. in flasks that were coated with black Scotch duct tape. After the bacteria reached an optical density (600 nm) of 0.4, a mix of IPTG, anhydrotetracycline, arabinose and hydroxocobalamin hydrochloride was added to the culture, giving final concentrations of 1 mM, 100 ng/ml, 0.2%, and 20 μM, respectively. Twenty-four hours later, the bacteria were harvested for coenzyme A analysis. Methylmalonyl-CoA production is illustrated in FIG. **24**. Host cells producing exogenous methylmalonyl-CoA mutase and methylmalonyl-CoA epimerase (encoded by pZA31 mutAB Ss epi) produced over 25 ng methylmalonyl-CoA per ml culture. Host cells comprising additional copies of the Sbm (methylmalonyl-CoA mutase) coding sequence produced over three times the amount of methylmalonyl-CoA per ml of culture, and co-expression of an methylmalonyl-CoA acyl transferase reduced the amount of methylmalonyl-CoA present in the culture medium.

[0250] Production of methylmalonyl-CoA in host cells expressing exogenous propionyl-CoA carboxylase also was studied and is illustrated in FIG. **25**. BW25113 (control) and BW25113 containing pZA31-accA1-pccB (labeled as Pcc in the figure) were cultured in LB, and the coenzyme-A thioesters were isolated and characterized as described above. Host cells comprising a polynucleotide encoding an exogenous propionyl-CoA carboxylase produced over about 15 ng methylmalonyl-CoA per ml of culture.

[0251] When Ec 'tesA was present, less longer-chain (fifteen and seventeen carbons) and more mid-chain (thirteen carbons) branched fatty acids were produced by the host cell, indicating that production of thioesterase increases the proportion of medium chain-length branched fatty acids produced by the inventive method.

Example 16

Analysis of Scattered Branched Fatty Acid by Two-Dimensional (2D) Gas Chromatography

[0252] To identify branched fatty acids produced by recombinant *E. coli* produced as described herein, fatty acids were isolated from bacterial cultures and derivatives were generated to facilitate identification. The fatty acid derivatives were separated by 2D gas chromatography and mass spectrometry was used to characterize fragmented samples. Derivatization of fatty acids to their 4,4' dimethyloxazoline derivatives prior to analysis via mass spectrometry has been described (Zhang, J. Y., QT. Yu, B. N. Liu and Z. H. Huang, *Biomed Env. Mass Spectrom.* 15:33 (1988)). By careful examination of minor spectral differences, it possible to determine the location of branch points on the backbones of fatty acid derivatives.

[0253] One liter of bacterial samples in LB (modified to contain only 0.5 mg/ml sodium chloride, unless otherwise indicated) with cyanocobalamin (20 μM) were cultured at 22° C. for 25 hours following induction with IPTG, anhydrotetracycline, and arabinose. A cell pellet was collected by centrifugation at 3500 rpm, and the supernatant was discarded. The cell pellet was suspended in the remaining liquid, and the slurry was transferred into Pyrex tubes (#9826, Corning Inc., Lowell, Mass.). An equal volume of chloroform was added, and the sample was dried at room temperature overnight.

[0254] To produce samples for analysis, cell pellets (0.5 grams) were placed in a round bottom flask, and 0.5 grams of KOH pellets and 25 ml of water were added. The *E. coli* pellets and KOH solution were refluxed for three hours, and the sample was allowed to cool. Concentrated HCl was added drop-wise, using a methyl orange endpoint to ensure fatty carboxylic acids were in the acid form. The acidified aqueous solution was then extracted three times with 25 ml aliquots of hexane to extract the fatty acids into the organic layer.

[0255] To convert fatty acid to oxazoline derivatives, the hexane extract was evaporated to dryness and reconstituted

into 5 ml of hexane to which sodium sulfate was added as a drying agent. After evaporating the sample to a 1 ml volume, a portion (0.6 ml) was decanted into a Reactitherm™ vial. The hexane in the Reactitherm™ vial was again evaporated to dryness, and 2 ml of 2-methyl-2-aminopropanol was added. The vial was capped and heated for 4 hours at 200° C. The cooled 2-methyl-2-aminopropanol solution was transferred to a scintillation vial, to which 5 ml of methylene chloride was added. The sample was washed with three 5 ml volumes of water. Sodium sulfate was added to the methylene chloride to remove any residual water, and an aliquot was transferred to a GC vial for analysis.

[0256] The derivatized samples were analyzed on a Leco Pegasus 4D Comprehensive 2D gas chromatograph time-of-flight mass spectrometer equipped with a 30M Supelco GammaDex 120 (Supelco 24307) column in the first dimension and a 2M Varian VF5-MS (Varian CP9034) column in the second dimension. Retention times of key chain-length fatty acids (in both first and second dimensions) in test samples were confirmed by identical preparation and analysis of a Supleco (47080-U) BAME (bacterial acid methyl ester) standard mixture. Using these columns, 4,4' dimethyloxazoline-derivatized branched-chain fatty acids were expected to elute prior to their linear chain-length homologs in the first dimension, and this was confirmed by the iso and anteiso structural isomers of C15 methyl esters (derivatized to their 4,4'-dimethyloxazoline derivatives) in the BAME standard reference above.

[0257] The profile of fatty acids produced by two strains was compared. The first strain was engineered to produce branched fatty acids [BL21 Star (DE3) (pTrcHisA Ec sbm So ce epi pZA31 mmat)] and the second was a control strain [BL21 Star (DE3) (pTrcHisA pZA31)]. A sample of *E. coli* BL21 Star (DE3) comprising pTrcHisA Ec sbm So ce epi and pZA31 mmat was deposited with American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Va., on Dec. 14, 2010, under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure ("Budapest Treaty"), and assigned Deposit Accession No. [XXX] on [DATE]. The sample from the first strain revealed several peaks in the region where branched fatty acids were expected (FIG. **26**), whereas the sample from the control strain revealed no such peaks (FIG. **27**). For example, several peaks (labeled 54, 55, and 57) were in a position consistent with branched C15 acids, and peaks 137 and 139 were in a position expected for branched C17 acids. Mass spectrometry established that these peaks comprise branched fatty acids.

[0258] The mass spectral fragmentation pattern of oxazoline derivatives was used to confirm that the fatty acids identified using 2D GC contained branches. Oxazoline derivatives fragment along the length of the carbon chain starting from the functional end of the molecule. If a branch point occurs along the backbone, there is a gap in the mass spectrum pattern; which peak is missing (or reduced) depends on the location of the branch. FIG. **28** depicts the mass spectra of the peaks labeled 54, 55, and 57 in FIG. **26** as oxazoline derivatives of methyl-branched tetradecanoic fatty acids. The ions circled exhibit reduced or no intensity relative to the reference spectrum of linear pentadecanoic fatty acid (bottom spectrum), and were assigned as 8-methyl, 10-methyl, and 12-methyl (anteiso) tetradecanoic fatty acid (all as oxazoline derivatives). Peak 57 was tentatively identified as the anteiso C15 oxazoline derivative despite the similarity to the mass spec data for the linear sample because 1) peak 61 migrated at the position of an anteiso C15 standard on 2D gas chromatography, 2) the 252 molecular weight ion is present in slightly lower amounts relative to the nearby 238 and 266 molecular weight ions, and 3) anteiso compounds can be difficult to identify by this technique. The 8- and 10-branched fatty acids are shown in the top two profiles of FIG. **28**, readily identified by the almost complete absence of the fragment circled. Peaks 137 and 139 in FIG. **26** were assigned as 8-methylhexadecanoic acid and 12-methylhexadecanoic acids (as oxazoline derivatives). Thus, B132 Star (DE3) (pTrcHisA Ec sbm So ce epi pZA31 mmat) (i.e., a recombinant microbe comprising overexpressed or recombinant polynucleotides encoding a methylmalonyl-CoA mutase, a methylmalonyl-CoA epimerase, and an acyl transferase) generated branched-chain C15 and C17 fatty acids comprising methyl branches on even-number carbons.

[0259] Branched fatty acid production also was observed in host cells producing exogenous propionyl-CoA carboxylase and *Streptomyces coelicolor* methylmalonyl-CoA mutase. The propionyl-CoA carboxylase gene-containing strain produced the branched fatty acids shown in Table F.

TABLE F

Peak #	Proposed Compound ID	Formula	Molecular Weight DMOX	Molecular Weight as fatty acid
38	6-methyl, dodecanoic acid (DMOX)	$C_{13}H_{33}$ (C_4H_8NO)	267	214
40	8-methyl, dodecanoic acid (DMOX)	$C_{13}H_{33}$ (C_4H_8NO)	267	214
61	6-methyl, tridecanoic acid (DMOX)	$C_{14}H_{35}$ (C_4H_8NO)	281	228
62	8-methyl, tridecanoic acid (DMOX)	$C_{14}H_{35}$ (C_4H_8NO)	281	228
101	6-methyl, tetradecanoic acid (DMOX)	$C_{15}H_{37}$ (C_4H_8NO)	295	242
103	10-methyl, tetradecanoic acid (DMOX)	$C_{15}H_{37}$ (C_4H_8NO)	295	242
140	10-methyl, pentadecanoic acid (DMOX)	$C_{16}H_{39}$ (C_4H_8NO)	309	256
182	8-methyl, hexadecanoic acid (DMOX)	$C_{17}H_{41}$ (C_4H_8NO)	323	270
189	12-methyl, hexadecanoic acid (DMOX)	$C_{17}H_{41}$ (C_4H_8NO)	323	270

[0260] The *S. coelicolor* methylmalonyl-CoA mutase gene-containing microbe (BL21 Star (DE3) harboring pZA31 mutAB Ss epi pTrcHisA mmat) produced four branched fatty acids: 6-methyltetradecanoic acid, 10-methyltetradecanoic acid, 6-methylhexadecanoic acid, and 12-methylhexadecanoic acid.

[0261] Using 2D gas chromatography and mass spectrometry, fatty acid profiles were compared for two recombinant strains comprising Ec sbm, So ce epi, Mb mmat and containing or lacking a thioesterase coding sequence ('tesA). The amount of branched C15 fatty acids relative to branched C17 fatty acids was greater in the 'tesA-containing strain. The area percent ratio of branched C15 fatty acid to branched C17 fatty acids in K27-Z1 (pTrcHisA Ec sbm So ce epi pZA31 mmat) was 1.4, while the ratio produced by K27-Z1 (pTrcHisA Ec sbm So ce epi pZA31 mmat pZS22 Ec 'tesA) was 7.0. Expression of a thioesterase shortened the chain length of branched fatty acids.

[0262] These results demonstrate that a cell of the invention producing propionyl-CoA carboxylase or producing methyl-

malonyl-CoA mutase, methylmalonyl-CoA epimerase, and acyl transferase generates branched-chain fatty acids comprising methyl branches on even-number carbons. Recombinant host cells further comprising a polynucleotide encoding a thioesterase preferentially produce fatty acid comprising shorter chain length.

[0263] The dimensions and values disclosed herein are not to be understood as being strictly limited to the exact numerical values recited. Instead, unless otherwise specified, each such dimension is intended to mean both the recited value and a functionally equivalent range surrounding that value. For example, a dimension disclosed as "40 mm" is intended to mean "about 40 mm."

[0264] Every document cited herein, including any cross referenced or related patent or application, is hereby incorporated herein by reference in its entirety unless expressly excluded or otherwise limited. The citation of any document is not an admission that it is prior art with respect to any invention disclosed or claimed herein or that it alone, or in any combination with any other reference or references, teaches, suggests or discloses any such invention. Further, to the extent that any meaning or definition of a term in this document conflicts with any meaning or definition of the same term in a document incorporated by reference, the meaning or definition assigned to that term in this document shall govern.

[0265] While particular embodiments of the invention have been illustrated and described, it would be obvious to those skilled in the art that various other changes and modifications can be made without departing from the spirit and scope of the invention. It is therefore intended to cover in the appended claims all such changes and modifications that are within the scope of this invention.

SEQUENCE LISTING

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aaagttctga accgtggccg tccgccggag aaacagctga cgtttgcgga atgtctgaaa 180

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gcgccgaaga aactgggtta cccaggcgtt gccccattta cacgtgggac cacggttcgt 300

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tatcataatg cgggtgcggg cgatgtagca gagctggctt gggccctggc taccggtgcg 780

gaatatgttc gtgcactggt agaacaaggt tttacggcga ccgaggcgtt cgatacgatt 840

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gccgcaccgg cccgtaaagg tctggcatgg catcgcgaca gtgaagtatt cgaacaactg 1500

atggatcgca gcaccagtgt gagtgaacgt ccaaaggttt tcctggcgtg cctgggcaca 1560

cgtcgtgact tcggtggtcg tgagggtttt agcagcccag tgtggcatat cgcaggcatt 1620

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ggggcacaag ttgcggatct gtgctctagc gccaaagtgt acgctcagca gggtctggag 1740

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gagttcggcg atgatgcggc tgaggcggag aaactgatcg atggtcgcct gtttatgggt 1860

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<212> TYPE: PRT
<213> ORGANISM: Janibacter sp. HTCC2649

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35 40 45

Arg Gly Glu Val Gly Lys Val Gly Val Pro Ile Ser His Ile Gly Asp
50 55 60

Met Arg Ala Leu Phe Asp Gln Ile Pro Leu Gly Gln Met Asn Thr Ser
65 70 75 80

Met Thr Ile Asn Ala Thr Ala Met Trp Leu Leu Ala Met Tyr Gln Val
85 90 95

Ala Ala Glu Asp Gln Ala Thr Ala Ala Asp Glu Asp Pro Ala Ser Val
100 105 110

Val Lys Ala Leu Gly Gly Thr Thr Gln Asn Asp Ile Ile Lys Glu Tyr
115 120 125

Leu Ser Arg Gly Thr Tyr Val Phe Ala Pro Ala Pro Ser Leu Arg Leu
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Ile Thr Asp Met Val Ser Tyr Thr Val Ser Asp Ile Pro Lys Trp Asn
145 150 155 160

Pro Ile Asn Ile Cys Ser Tyr His Leu Gln Glu Ala Gly Ala Thr Pro
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Val Gln Glu Ile Ala Tyr Ala Met Ser Thr Ala Ile Ala Val Leu Asp
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Ala Val Arg Asp Ala Gly Gln Val Pro Gln Glu Arg Phe Gly Glu Val
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Val Ala Arg Ile Ser Phe Phe Val Asn Ala Gly Val Arg Phe Val Glu
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            260                 265                 270

Asn Val Gln Arg Ile Val Leu Glu Met Leu Ala Val Thr Leu Ser Lys
        275                 280                 285

Gly Ala Arg Ala Arg Ala Val Gln Leu Pro Ala Trp Asn Glu Ala Leu
    290                 295                 300

Gly Leu Pro Arg Pro Trp Asp Gln Gln Trp Ser Leu Arg Met Gln Gln
305                 310                 315                 320

Val Leu Ala Tyr Glu Ser Asp Leu Leu Glu Tyr Glu Asp Leu Phe Glu
                325                 330                 335

Gly Ser Ala Val Val Glu Ala Lys Val Ala Glu Leu Val Ala Gly Ala
            340                 345                 350

Lys Ala Glu Ile Ala Arg Val Ala Glu Leu Gly Gly Ala Val Ala Ala
        355                 360                 365

Val Glu Ser Gly Tyr Met Lys Ser Ala Leu Val Ala Ser His Ala Leu
    370                 375                 380

Arg Arg Gln Arg Ile Glu Ala Gly Glu Asp Ile Val Val Gly Val Asn
385                 390                 395                 400

Lys Phe Glu Thr Thr Glu Pro Asn Pro Leu Thr Ala Asp Leu Asp Thr
                405                 410                 415

Ala Ile Gln Ser Val Asp Ala Gly Val Glu Ala Ala Ala Ala Lys Ala
            420                 425                 430

Val Arg Glu Trp Arg Glu Thr Arg Asp Ala Asp Pro Val Lys Arg Glu
        435                 440                 445

Arg Ala Val Ala Ala Leu Ala Arg Leu Lys Ala Ala Ala Gln Thr Asp
    450                 455                 460

Glu Asn Leu Met Glu Ala Ser Ile Glu Cys Ala Arg Ala Glu Val Thr
465                 470                 475                 480

Thr Gly Glu Trp Ala Gln Ala Leu Arg Glu Val Phe Gly Glu Phe Arg
                485                 490                 495

Ala Pro Thr Gly Val Thr Gly Thr Val Gly Leu Thr Gly Gly Ala Ala
            500                 505                 510

Gly Ala Glu Leu Ser Ala Val Arg Glu Arg Val Ala Gly Leu Arg Asp
        515                 520                 525

Glu Leu Gly Glu Thr Leu Arg Val Leu Val Gly Lys Pro Gly Leu Asp
    530                 535                 540

Gly His Ser Asn Gly Ala Glu Gln Ile Ala Val Arg Ala Arg Asp Ala
545                 550                 555                 560

Gly Phe Glu Val Ile Tyr Gln Gly Ile Arg Leu Thr Pro Glu Gln Ile
                565                 570                 575

Val Ala Ala Ala Val Ser Glu Asp Val His Leu Val Gly Ile Ser Ile
            580                 585                 590

Leu Ser Gly Ser His Met Glu Leu Ile Pro Glu Val Leu Asp Arg Leu
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Arg Glu Ala Gly Ala Gly Asp Ile Pro Val Ile Val Gly Gly Ile Ile
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<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

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Val Asn Lys Gly Arg Pro Glu Asp Gln His Leu Ser Gly Asp Asp Ala
        35                  40                  45

Val Ala Thr Met Arg Ser His Leu Glu Gly Gly Leu Asp Ile Glu Pro
    50                  55                  60

Leu Tyr Met Lys Ser Ser Asp Pro Val Pro Leu Gly Val Pro Gly Ala
65                  70                  75                  80

Met Pro Phe Thr Arg Gly Arg Ala Leu Arg Asp Ala Asp Val Pro Trp
                85                  90                  95

Asp Val Arg Gln Val His Asp Asp Pro Asp Ala Ala Ala Thr Arg Gln
            100                 105                 110

Leu Val Leu Ala Asp Leu Glu Asn Gly Val Thr Ser Val Trp Leu His
        115                 120                 125

Val Gly Ala Asp Gly Leu Ala Pro Asn Asp Val Ala Glu Ala Leu Ala
    130                 135                 140

Glu Val Arg Leu Glu Leu Ala Pro Val Val Val Ser Ser Trp Asp Asp
145                 150                 155                 160

Gln Thr Ala Ala Ala Asp Ala Leu Tyr Ala Val Leu Ser Gly Ser Arg
                165                 170                 175

Ala Ser Ser Gly Asn Leu Gly His Asp Pro Leu Gly Ala Ala Ala Arg
            180                 185                 190

Thr Gly Ser Ala Pro Asp Leu Ala Pro Leu Ala Asp Ala Val Arg Arg
        195                 200                 205

Leu Ala Asp His Gly Glu Ile Arg Ala Ile Thr Val Asp Thr Arg Val
    210                 215                 220

His Gly Asp Ala Gly Val Thr Val Thr Asp Glu Val Ala Phe Ala Leu
225                 230                 235                 240

Ala Thr Gly Val Ala Tyr Leu Arg His Leu Glu Ser Glu Gly Val Asp
                245                 250                 255

Val Ala Glu Ala Phe Arg Asn Ile Glu Phe Arg Val Ser Ala Thr Ala
            260                 265                 270

Asp Gln Phe Leu Thr Ala Ala Ala Leu Arg Ala Leu Arg Arg Ala Trp
        275                 280                 285

Ala Arg Ile Gly Glu Ser Val Gly Val Pro Glu Thr Ser Arg Gly Ala
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Phe Thr His Ala Val Thr Ser Gly Arg Ile Phe Thr Arg Asp Asp Ala
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Leu Pro Thr Pro Phe Ser Arg Arg Ile Ala Arg Asn Thr Gln Ile Leu
        355                 360                 365

Leu Ala Glu Glu Ser Asn Val Ala Arg Val Thr Asp Pro Ala Gly Gly
    370                 375                 380

Ser Trp Tyr Val Glu Thr Leu Thr Asp Asp Val Ala Lys Ala Ala Trp
385                 390                 395                 400

Glu Thr Phe Gln Glu Ile Glu Ser Ala Gly Gly Met Val Ala Ala Leu
                405                 410                 415

Ala Asn Gly Leu Val Ala Gln Arg Ile Leu Ala Ala Val Ala Glu Arg
            420                 425                 430

Asp Ala Ala Leu Ala Thr Arg Ser Thr Pro Ile Thr Gly Val Ser Thr
        435                 440                 445

Phe Pro Leu Ala Gly Glu Lys Pro Leu Glu Arg Val Val Arg Ala Glu
    450                 455                 460

Leu Pro Val Gln Pro Asn Ala Leu Ala Pro His Arg Asp Ser Ala Ile
465                 470                 475                 480

Phe Glu Ala Leu Arg Asp Arg Ser Ala Ala Tyr Ala Thr Glu His Gly
                485                 490                 495

His Ala Pro Arg Val Ser Val Pro Thr Leu Asp Val Pro Arg Ala Ala
            500                 505                 510

Asp Arg Arg Ile Asp Ala Val Asn Leu Leu Thr Val Ala Gly Ile Asp
        515                 520                 525

Ala Val Asp Gly Asp Thr Glu Ser Ala Ala Ala Leu Thr Gly Thr Asp
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Ser Glu Val Asn Glu Glu Gln Gly Val Arg Glu Ala Met Leu Lys Ile
        35                  40                  45

Asn Glu Thr Ser Asp Gly Gly Ala Ser Tyr Leu Gln Leu Leu Glu Pro
    50                  55                  60

Thr Arg Pro Asp Ser Thr Val Ala Lys Trp Leu Asp Lys Asn Gly Glu
65                  70                  75                  80

Gly Val His His Ile Ala Phe Gly Thr Ala Asp Val Asp Gln Asp Ala
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Ala Asp Ile Lys Asp Lys Gly Val Arg Val Leu Tyr Glu Glu Pro Arg
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Arg Gly Ser Met Gly Ser Arg Ile Thr Phe Leu His Pro Lys Asp Cys
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Glu His
145


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<212> TYPE: PRT
<213> ORGANISM: Streptomyces sviceus

<400> SEQUENCE: 6

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            20                  25                  30

Ser Glu Val Asn Glu Glu Gln Gly Val Arg Glu Ala Met Leu Lys Ile
        35                  40                  45

Asn Glu Thr Ser Asp Gly Gly Ala Ser Tyr Leu Gln Leu Leu Glu Pro
    50                  55                  60

Thr Arg Pro Asp Ser Thr Val Ala Lys Trp Leu Asp Lys Asn Gly Glu
65                  70                  75                  80

Gly Val His His Ile Ala Phe Gly Thr Ala Asp Val Asp Gln Asp Ala
                85                  90                  95

Ala Asp Ile Lys Asp Lys Gly Val Arg Val Leu Tyr Glu Glu Pro Arg
            100                 105                 110

Arg Gly Ser Met Gly Ser Arg Ile Thr Phe Leu His Pro Lys Asp Cys
        115                 120                 125

His Gly Val Leu Thr Glu Leu Val Thr Ser Ala Pro Val Glu Ser Pro
    130                 135                 140

Glu His
145


<210> SEQ ID NO 7
<211> LENGTH: 1773
<212> TYPE: DNA
<213> ORGANISM: Streptomyces coelicolor
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: GenBank / AF113603.1
<309> DATABASE ENTRY DATE: 1999-12-08
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(1773)

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gtcgtcgtct ccacccgcga ctgctccctc cagcgccgcc accaaaagct cgtcgaggag 720

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ggcacgatct tcttcctgga ggtcaacacc cgcctccagg tcgagcaccc ggtcaccgag 900

gaagtcgccg gcatcgactt ggtccgcgag atgttccgca tcgccgacgg cgaggaactc 960

ggttacgacg accccgccct gcgcggccac tccttcgagt tccgcatcaa cggcgaggac 1020

cccggccgcg gcttcctgcc cgccccggc accgtcaccc tcttcgacgc gcccaccggc 1080

cccggcgtcc gcctggacgc cggcgtcgag tccggctccg tcatcggccc cgcctgggac 1140

tccctcctcg ccaaactgat cgtcaccggc cgcacccgcg ccgaggcact ccagcgcgcg 1200

gcccgcgccc tggacgagtt caccgtcgag ggcatggcca ccgccatccc cttccaccgc 1260

acggtcgtcc gcgacccggc cttcgccccc gaactcaccg gctccacgga ccccttcacc 1320

gtccacaccc ggtggatcga gacggagttc gtcaacgaga tcaagccctt caccacgccc 1380

gccgacaccg agacggacga ggagtcgggc cgggagacgg tcgtcgtcga ggtcggcggc 1440

aagcgcctgg aagtctccct cccctccagc ctgggcatgt ccctggcccg caccggcctg 1500

gccgccgggg cccgccccaa gcgccgcgcg gccaagaagt ccggccccgc cgcctcgggc 1560

gacaccctcg cctccccgat gcagggcacg atcgtcaaga tcgccgtcga ggaaggccag 1620

gaagtccagg aaggcgacct catcgtcgta ctcgaggcga tgaagatgga acagcccctc 1680

aacgcccaca ggtccggcac catcaagggc ctcaccgccg aggtcggcgc ctccctcacc 1740

tccggcgccg ccatctgcga gatcaaggac tga 1773

<210> SEQ ID NO 8
<211> LENGTH: 1593
<212> TYPE: DNA
<213> ORGANISM: Streptomyces coelicolor
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: GenBank / AF113605.1
<309> DATABASE ENTRY DATE: 1999-12-08
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(1593)

<400> SEQUENCE: 8

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cacgccaagg gcaagctgac ggctcgtgaa cgcatcgacc tcctcctcga cgagggttcc 180

ttcgtcgagc tggacgagtt cgcccggcac cgctccacca acttcggcct cgacgccaac 240

cgcccctacg gcgacggcgt cgtcaccggc tacggcaccg tcgacggccg ccccgtggcc 300

gtcttctccc aggacttcac cgtcttcggc ggcgcgctgg gcgaggtcta cggccagaag 360

atcgtcaagg tgatggactt cgccctcaag accggctgcc cggtcgtcgg catcaacgac 420

tccggcggcg cccgcatcca ggagggcgtg gcctccctcg gcgcctacgg cgagatcttc 480

cgccgcaaca cccacgcctc cggcgtgatc ccgcagatca gcctggtcgt cggcccgtgt 540

gcgggcggcg cggtgtactc ccccgcgatc accgacttca cggtgatggt ggaccagacc 600

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agccacatgt tcatcaccgg tcccgacgtc atcaagacgg tcaccggcga ggacgtcggc    660

ttcgaggagc tgggcggcgc ccgcacccac aactccacct cgggcgtggc ccaccacatg    720

gccggcgacg agaaggacgc ggtcgagtac gtcaagcagc tcctgtcgta cctgccgtcc    780

aacaacctct ccgagccccc cgccttcccg gaggaggcgg acctcgcggt cacggacgag    840

gacgccgagc tggacacgat cgtcccggac tcggcgaacc agccctacga catgcactcc    900

gtcatcgagc acgtcctgga cgacgccgag ttcttcgaga cgcaacccct cttcgcgccg    960

aacatcctca ccggcttcgg ccgcgtggag ggccgcccgg tcggcatcgt cgccaaccag   1020

cccatgcagt tcgccggctg cctggacatc acggcctccg agaaggcggc ccgcttcgtg   1080

cgcacctgcg acgccttcaa cgtccccgtc ctcaccttcg tggacgtccc cggcttcctg   1140

cccggcgtcg accaggagca cgacggcatc atccgccgcg gcgccaagct gatcttcgcc   1200

tacgccgagg ccacggtgcc gctcatcacg gtcatcaccc gcaaggcctt cggcggcgcc   1260

tacgacgtca tgggctccaa gcacctgggc gccgacctca acctggcctg gcccaccgcc   1320

cagatcgccg tcatgggcgc ccaaggcgcg gtcaacatcc tgcaccgccg caccatcgcc   1380

gacgccggtg acgacgccga ggccacccgg gcccgcctga tccaggagta cgaggacgcc   1440

ctcctcaacc cctacacggc ggccgaacgc ggctacgtcg acgccgtgat catgccctcc   1500

gacactcgcc gccacatcgt ccgcggcctg cgccagctgc gcaccaagcg cgagtccctg   1560

cccccgaaga agcacggcaa catccccctg taa                                1593
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<210> SEQ ID NO 9
<211> LENGTH: 590
<212> TYPE: PRT
<213> ORGANISM: Streptomyces coelicolor
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: GenBank / AF113603.1
<309> DATABASE ENTRY DATE: 1999-12-08
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(590)

<400> SEQUENCE: 9

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Met Arg Lys Val Leu Ile Ala Asn Arg Gly Glu Ile Ala Val Arg Val
1               5                   10                  15

Ala Arg Ala Cys Arg Asp Ala Gly Ile Ala Ser Val Ala Val Tyr Ala
            20                  25                  30

Asp Pro Asp Arg Asp Ala Leu His Val Arg Ala Ala Asp Glu Ala Phe
        35                  40                  45

Ala Leu Gly Gly Asp Thr Pro Ala Thr Ser Tyr Leu Asp Ile Ala Lys
    50                  55                  60

Val Leu Lys Ala Ala Arg Glu Ser Gly Ala Asp Ala Ile His Pro Gly
65                  70                  75                  80

Tyr Gly Phe Leu Ser Glu Asn Ala Glu Phe Ala Gln Ala Val Leu Asp
                85                  90                  95

Ala Gly Leu Ile Trp Ile Gly Pro Pro Pro His Ala Ile Arg Asp Arg
            100                 105                 110

Gly Glu Lys Val Ala Ala Arg His Ile Ala Gln Arg Ala Gly Ala Pro
        115                 120                 125

Leu Val Ala Gly Thr Pro Asp Pro Val Ser Gly Ala Asp Glu Val Val
    130                 135                 140

Ala Phe Ala Lys Glu His Gly Leu Pro Ile Ala Ile Lys Ala Ala Phe
145                 150                 155                 160
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```
Gly Gly Gly Gly Arg Gly Leu Lys Val Ala Arg Thr Leu Glu Glu Val
                165                 170                 175

Pro Glu Leu Tyr Asp Ser Ala Val Arg Glu Ala Val Ala Ala Phe Gly
            180                 185                 190

Arg Gly Glu Cys Phe Val Glu Arg Tyr Leu Asp Lys Pro Arg His Val
        195                 200                 205

Glu Thr Gln Cys Leu Ala Asp Thr His Gly Asn Val Val Val Val Ser
    210                 215                 220

Thr Arg Asp Cys Ser Leu Gln Arg Arg His Gln Lys Leu Val Glu Glu
225                 230                 235                 240

Ala Pro Ala Pro Phe Leu Ser Glu Ala Gln Thr Glu Gln Leu Tyr Ser
                245                 250                 255

Ser Ser Lys Ala Ile Leu Lys Glu Ala Gly Tyr Gly Gly Ala Gly Thr
            260                 265                 270

Val Glu Phe Leu Val Gly Met Asp Gly Thr Ile Phe Phe Leu Glu Val
        275                 280                 285

Asn Thr Arg Leu Gln Val Glu His Pro Val Thr Glu Glu Val Ala Gly
    290                 295                 300

Ile Asp Leu Val Arg Glu Met Phe Arg Ile Ala Asp Gly Glu Glu Leu
305                 310                 315                 320

Gly Tyr Asp Asp Pro Ala Leu Arg Gly His Ser Phe Glu Phe Arg Ile
                325                 330                 335

Asn Gly Glu Asp Pro Gly Arg Gly Phe Leu Pro Ala Pro Gly Thr Val
            340                 345                 350

Thr Leu Phe Asp Ala Pro Thr Gly Pro Gly Val Arg Leu Asp Ala Gly
        355                 360                 365

Val Glu Ser Gly Ser Val Ile Gly Pro Ala Trp Asp Ser Leu Leu Ala
    370                 375                 380

Lys Leu Ile Val Thr Gly Arg Thr Arg Ala Glu Ala Leu Gln Arg Ala
385                 390                 395                 400

Ala Arg Ala Leu Asp Glu Phe Thr Val Glu Gly Met Ala Thr Ala Ile
                405                 410                 415

Pro Phe His Arg Thr Val Val Arg Asp Pro Ala Phe Ala Pro Glu Leu
            420                 425                 430

Thr Gly Ser Thr Asp Pro Phe Thr Val His Thr Arg Trp Ile Glu Thr
        435                 440                 445

Glu Phe Val Asn Glu Ile Lys Pro Phe Thr Thr Pro Ala Asp Thr Glu
    450                 455                 460

Thr Asp Glu Glu Ser Gly Arg Glu Thr Val Val Val Glu Val Gly Gly
465                 470                 475                 480

Lys Arg Leu Glu Val Ser Leu Pro Ser Ser Leu Gly Met Ser Leu Ala
                485                 490                 495

Arg Thr Gly Leu Ala Ala Gly Ala Arg Pro Lys Arg Arg Ala Ala Lys
            500                 505                 510

Lys Ser Gly Pro Ala Ala Ser Gly Asp Thr Leu Ala Ser Pro Met Gln
        515                 520                 525

Gly Thr Ile Val Lys Ile Ala Val Glu Glu Gly Gln Glu Val Gln Glu
    530                 535                 540

Gly Asp Leu Ile Val Val Leu Glu Ala Met Lys Met Glu Gln Pro Leu
545                 550                 555                 560
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```
Asn Ala His Arg Ser Gly Thr Ile Lys Gly Leu Thr Ala Glu Val Gly
                565                 570                 575

Ala Ser Leu Thr Ser Gly Ala Ala Ile Cys Glu Ile Lys Asp
            580                 585                 590


<210> SEQ ID NO 10
<211> LENGTH: 530
<212> TYPE: PRT
<213> ORGANISM: Streptomyces coelicolor
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: GenBank / AF113605.1
<309> DATABASE ENTRY DATE: 1999-12-08
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(530)

<400> SEQUENCE: 10

Met Ser Glu Pro Glu Glu Gln Gln Pro Asp Ile His Thr Thr Ala Gly
1               5                   10                  15

Lys Leu Ala Asp Leu Arg Arg Arg Ile Glu Glu Ala Thr His Ala Gly
            20                  25                  30

Ser Ala Arg Ala Val Glu Lys Gln His Ala Lys Gly Lys Leu Thr Ala
        35                  40                  45

Arg Glu Arg Ile Asp Leu Leu Leu Asp Glu Gly Ser Phe Val Glu Leu
    50                  55                  60

Asp Glu Phe Ala Arg His Arg Ser Thr Asn Phe Gly Leu Asp Ala Asn
65                  70                  75                  80

Arg Pro Tyr Gly Asp Gly Val Val Thr Gly Tyr Gly Thr Val Asp Gly
                85                  90                  95

Arg Pro Val Ala Val Phe Ser Gln Asp Phe Thr Val Phe Gly Gly Ala
            100                 105                 110

Leu Gly Glu Val Tyr Gly Gln Lys Ile Val Lys Val Met Asp Phe Ala
        115                 120                 125

Leu Lys Thr Gly Cys Pro Val Val Gly Ile Asn Asp Ser Gly Gly Ala
    130                 135                 140

Arg Ile Gln Glu Gly Val Ala Ser Leu Gly Ala Tyr Gly Glu Ile Phe
145                 150                 155                 160

Arg Arg Asn Thr His Ala Ser Gly Val Ile Pro Gln Ile Ser Leu Val
                165                 170                 175

Val Gly Pro Cys Ala Gly Gly Ala Val Tyr Ser Pro Ala Ile Thr Asp
            180                 185                 190

Phe Thr Val Met Val Asp Gln Thr Ser His Met Phe Ile Thr Gly Pro
        195                 200                 205

Asp Val Ile Lys Thr Val Thr Gly Glu Asp Val Gly Phe Glu Glu Leu
    210                 215                 220

Gly Gly Ala Arg Thr His Asn Ser Thr Ser Gly Val Ala His His Met
225                 230                 235                 240

Ala Gly Asp Glu Lys Asp Ala Val Glu Tyr Val Lys Gln Leu Leu Ser
                245                 250                 255

Tyr Leu Pro Ser Asn Asn Leu Ser Glu Pro Pro Ala Phe Pro Glu Glu
            260                 265                 270

Ala Asp Leu Ala Val Thr Asp Glu Asp Ala Glu Leu Asp Thr Ile Val
        275                 280                 285

Pro Asp Ser Ala Asn Gln Pro Tyr Asp Met His Ser Val Ile Glu His
    290                 295                 300

Val Leu Asp Asp Ala Glu Phe Phe Glu Thr Gln Pro Leu Phe Ala Pro
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305                 310                 315                 320

Asn Ile Leu Thr Gly Phe Gly Arg Val Glu Gly Arg Pro Val Gly Ile
                325                 330                 335

Val Ala Asn Gln Pro Met Gln Phe Ala Gly Cys Leu Asp Ile Thr Ala
            340                 345                 350

Ser Glu Lys Ala Ala Arg Phe Val Arg Thr Cys Asp Ala Phe Asn Val
        355                 360                 365

Pro Val Leu Thr Phe Val Asp Val Pro Gly Phe Leu Pro Gly Val Asp
    370                 375                 380

Gln Glu His Asp Gly Ile Ile Arg Arg Gly Ala Lys Leu Ile Phe Ala
385                 390                 395                 400

Tyr Ala Glu Ala Thr Val Pro Leu Ile Thr Val Ile Thr Arg Lys Ala
                405                 410                 415

Phe Gly Gly Ala Tyr Asp Val Met Gly Ser Lys His Leu Gly Ala Asp
            420                 425                 430

Leu Asn Leu Ala Trp Pro Thr Ala Gln Ile Ala Val Met Gly Ala Gln
        435                 440                 445

Gly Ala Val Asn Ile Leu His Arg Arg Thr Ile Ala Asp Ala Gly Asp
    450                 455                 460

Asp Ala Glu Ala Thr Arg Ala Arg Leu Ile Gln Glu Tyr Glu Asp Ala
465                 470                 475                 480

Leu Leu Asn Pro Tyr Thr Ala Ala Glu Arg Gly Tyr Val Asp Ala Val
                485                 490                 495

Ile Met Pro Ser Asp Thr Arg Arg His Ile Val Arg Gly Leu Arg Gln
            500                 505                 510

Leu Arg Thr Lys Arg Glu Ser Leu Pro Pro Lys Lys His Gly Asn Ile
        515                 520                 525

Pro Leu
    530
```

<210> SEQ ID NO 11
<211> LENGTH: 116
<212> TYPE: DNA
<213> ORGANISM: Streptomyces coelicolor

<400> SEQUENCE: 11

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aattgtgagc ggataacaat tgacattgtg agcggataac aagatactga gcacatcagc     60

aggacgcact gaccgaattc aataattttg tttaacttta agaaggagat atacat        116
```

<210> SEQ ID NO 12
<211> LENGTH: 1773
<212> TYPE: DNA
<213> ORGANISM: Streptomyces coelicolor

<400> SEQUENCE: 12

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atgcgcaaag tgctgattgc gaaccgtggt gaaatcgccg ttcgtgtggc acgcgcgtgt     60

cgtgatgcag gtattgcaag tgttgcggtg tatgccgatc cggatcgcga tgcgctgcat    120

gttcgtgcgg ccgatgaagc ctttgcactg ggcggtgata ccccggcaac gagctatctg    180

gatattgcaa aagtgctgaa agcagcgcgc gaaagcggtg cggatgccat ccatccgggc    240

tacggttttc tgtctgaaaa tgcagaattt gcacaggcgg ttctggatgc aggtctgatt    300

tggatcggtc cgccgccgca tgcaattcgt gatctgggcg ataaagtggc cgcacgccac    360
```

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atcgcccagc gtgcaggcgc gccgctggtt gcgggcaccc cggacccggt ttctggtgca 420

gatgaagtgg ttgcgtttgc caaagaacat ggcctgccga ttgcgatcaa agcagcattc 480

ggcggtggcg gtcgcggtct gaaagtggcc cgtaccctgg aagaagttcc ggaactgtat 540

gatagcgcag ttcgcgaagc ggtggcagcg tttggccgtg gtgaatgctt cgtggaacgc 600

tacctggata aaccgcgtca tgttgaaacc cagtgtctgg cggatacgca cggcaacgtg 660

gttgtggtta gcacccgcga ttgctctctg caacgtcgcc accagaaact ggtggaagaa 720

gcaccggcgc cgtttctgag cgaagcccag accgaacagc tgtatagctc tagtaaagcg 780

attctgaaag aagccggtta cgtgggcgcc ggtacggttg aatttctggt gggcatggat 840

ggcaccatta gctttctgga agttaacacc cgtctgcaag ttgaacatcc ggtgaccgaa 900

gaagttgcgg gcattgatct ggtgcgcgaa atgtttcgta tcgcagatgg cgaagaactg 960

ggttacgatg atccggcgct gcgcggtcac agctttgaat ttcgtattaa tggcgaagat 1020

ccgggccgtg gttttctgcc ggcgccgggc accgtgacgc tgttcgatgc accgaccggt 1080

ccgggcgttc gtctggatgc cggtgtggaa agtggtagcg ttattggccc ggcatgggat 1140

agcctgctgg cgaaactgat cgttaccggt cgtacgcgcg ccgaagcgct gcaacgtgca 1200

gcacgtgccc tggatgaatt taccgtggaa ggcatggcga cggccattcc gtttcatcgc 1260

accgtggttc gtgatccggc attcgcgccg gaactgaccg gctctaccga tccgttcacc 1320

gtgcacacgc gctggatcga aaccgaattt gttaacgaaa tcaaaccgtt caccacgccg 1380

gcggataccg aaacggatga agaaagtggt cgcgaaacgg tggttgtgga agtgggcggt 1440

aaacgtctgg aagtttctct gccgagcagc ctgggtatga gtctggcgcg taccggtctg 1500

gcggccggcg cccgtccgaa acgtcgcgca gcgaaaaaat ctggtccggc cgcaagcggt 1560

gataccctgg ccagtccgat gcagggcacg attgtgaaaa tcgcagtgga agaaggtcag 1620

gaagtgcagg aaggcgatct gattgttgtg ctggaagcga tgaaaatgga acagccgctg 1680

aatgcccatc gtagcggcac catcaaaggc ctgacggccg aagtgggtgc atctctgacc 1740

agtggcgcgg ccatttgcga aatcaaagat taa 1773

<210> SEQ ID NO 13
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Streptomyces coelicolor

<400> SEQUENCE: 13

agatctgcgg ccgcatctag aaataatttt gtttaacttt aagaaggaga tatattc 57

<210> SEQ ID NO 14
<211> LENGTH: 1593
<212> TYPE: DNA
<213> ORGANISM: Streptomyces coelicolor

<400> SEQUENCE: 14

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ctgcgtcgcc gtatcgaaga agcaacccat gcaggtagcg cacgtgcagt ggaaaaacag 120

cacgcgaaag gtaaactgac ggcccgcgaa cgtatcgatc tgctgctgga tgaaggcagt 180

tttgttgaac tggatgaatt tgcacgccac cgtagcacca actttggtct ggatgcgaat 240

cgcccgtatg gcgatggtgt ggttaccggt tacggtacgg tggatggtcg tccggtggca 300

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atcgtgaaag ttatggattt cgcgctgaaa acgggctgcc cggtggttgg tattaacgat 420

agcggcggtg cccgcatcca ggaaggtgtt gcctctctgg gcgcgtatgg cgaaatcttt 480

cgccgtaata cccatgcgag tggcgtgatt ccgcagatca gcctggtggt tggtccgtgt 540

gcgggcggtg ccgtttactc tccggccatt accgatttta cggtgatggt tgatcagacc 600

agtcacatgt tcattacggg cccggatgtg atcaaaaccg ttacgggcga agatgtgggt 660

tttgaagaac tgggcggtgc acgtacccac aacagcacgt ctggcgttgc gcatcacatg 720

gccggtgatg aaaaagatgc cgtggaatat gttaaacagc tgctgagtta cctgccgagc 780

aacaatctgt ctgaaccgcc ggcgttcccg gaagaagcag acctggcggt gaccgatgaa 840

gatgccgaac tggatacgat cgttccggat tctgcaaatc agccgtacga tatgcacagt 900

gtgattgaac acgttctgga tgatgcggaa tttttcgaaa cccagccgct gtttgccccg 960

aacattctga cgggtttcgg tcgtgtggaa ggtcgtccgg tgggtatcgt tgcaaatcag 1020

ccgatgcagt ttgcgggttg cctggatatt accgcctctg aaaaagcggc ccgctttgtg 1080

cgtacctgtg atgcgttcaa cgtgccggtt ctgacgtttg tggatgttcc gggcttcctg 1140

ccgggtgttg atcaggaaca tgatggcatt atccgccgtg gtgcgaaact gatttttgcg 1200

tatgccgaag caaccgtgcc gctgattacc gttatcacgc gcaaagcatt cggcggtgcg 1260

tacgatgtga tgggcagcaa acatctgggt gccgatctga acctggcatg gccgaccgca 1320

cagatcgcag tgatgggcgc gcagggtgcc gttaatattc tgcaccgccg taccatcgca 1380

gatgcaggtg atgatgcaga agcgacgcgc gcacgtctga ttcaggaata tgaagatgcg 1440

ctgctgaacc cgtataccgc agcggaacgt ggttacgtgg atgcggttat tatgccgagc 1500

gatacccgcc gtcatatcgt gcgtggtctg cgtcagctgc gtacgaaacg tgaatctctg 1560

ccgccgaaaa aacacggtaa tattccgctg taa 1593

<210> SEQ ID NO 15
<211> LENGTH: 3539
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic nucleotide

<400> SEQUENCE: 15

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aggacgcact gaccgaattc aataattttg tttaacttta agaaggagat atacatatgc 120

gcaaagtgct gattgcgaac cgtggtgaaa tcgccgttcg tgtggcacgc gcgtgtcgtg 180

atgcaggtat tgcaagtgtt gcggtgtatg ccgatccgga tcgcgatgcg ctgcatgttc 240

gtgcggccga tgaagccttt gcactgggcg gtgatacccc ggcaacgagc tatctggata 300

ttgcaaaagt gctgaaagca gcgcgcgaaa gcggtgcgga tgccatccat ccgggctacg 360

gttttctgtc tgaaaatgca gaatttgcac aggcggttct ggatgcaggt ctgatttgga 420

tcggtccgcc gccgcatgca attcgtgatc tgggcgataa agtggccgca cgccacatcg 480

cccagcgtgc aggcgcgccg ctggttgcgg gcaccccgga cccggtttct ggtgcagatg 540

aagtggttgc gtttgccaaa gaacatggcc tgccgattgc gatcaaagca gcattcggcg 600

gtggcggtcg cggtctgaaa gtggcccgta ccctggaaga agttccggaa ctgtatgata 660

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gcgcagttcg cgaagcggtg gcagcgtttg gccgtggtga atgcttcgtg gaacgctacc 720

tggataaacc gcgtcatgtt gaaacccagt gtctggcgga tacgcacggc aacgtggttg 780

tggttagcac ccgcgattgc tctctgcaac gtcgccacca gaaactggtg gaagaagcac 840

cggcgccgtt tctgagcgaa gcccagaccg aacagctgta tagctctagt aaagcgattc 900

tgaaagaagc cggttacgtg ggcgccggta cggttgaatt tctggtgggc atggatggca 960

ccattagctt tctggaagtt aacacccgtc tgcaagttga acatccggtg accgaagaag 1020

ttgcgggcat tgatctggtg cgcgaaatgt ttcgtatcgc agatggcgaa gaactgggtt 1080

acgatgatcc ggcgctgcgc ggtcacagct ttgaatttcg tattaatggc gaagatccgg 1140

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gtctggaagt ttctctgccg agcagcctgg gtatgagtct ggcgcgtacc ggtctggcgg 1620

ccggcgcccg tccgaaacgt cgcgcagcga aaaaatctgg tccggccgca agcggtgata 1680

ccctggccag tccgatgcag ggcacgattg tgaaaatcgc agtggaagaa ggtcaggaag 1740

tgcaggaagg cgatctgatt gttgtgctgg aagcgatgaa aatggaacag ccgctgaatg 1800

cccatcgtag cggcaccatc aaaggcctga cggccgaagt gggtgcatct ctgaccagtg 1860

gcgcggccat ttgcgaaatc aaagattaaa gatctgcggc cgcatctaga aataattttg 1920

tttaacttta agaaggagat atattcatga gtgaaccgga agaacagcag ccggatattc 1980

ataccacggc aggcaaactg gcggatctgc gtcgccgtat cgaagaagca acccatgcag 2040

gtagcgcacg tgcagtggaa aaacagcacg cgaaaggtaa actgacggcc cgcgaacgta 2100

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cactgggcga agtttacggt cagaaaatcg tgaaagttat ggatttcgcg ctgaaaacgg 2340

gctgcccggt ggttggtatt aacgatagcg gcggtgcccg catccaggaa ggtgttgcct 2400

ctctgggcgc gtatggcgaa atctttcgcc gtaataccca tgcgagtggc gtgattccgc 2460

agatcagcct ggtggttggt ccgtgtgcgg gcggtgccgt ttactctccg gccattaccg 2520

attttacggt gatggttgat cagaccagtc acatgttcat tacgggcccg gatgtgatca 2580

aaaccgttac gggcgaagat gtgggttttg aagaactggg cggtgcacgt acccacaaca 2640

gcacgtctgg cgttgcgcat cacatggccg gtgatgaaaa agatgccgtg gaatatgtta 2700

aacagctgct gagttacctg ccgagcaaca atctgtctga accgccggcg ttcccggaag 2760

aagcagacct ggcggtgacc gatgaagatg ccgaactgga tacgatcgtt ccggattctg 2820

caaatcagcc gtacgatatg cacagtgtga ttgaacacgt tctggatgat gcggaatttt 2880

tcgaaaccca gccgctgttt gccccgaaca ttctgacggg tttcggtcgt gtggaaggtc 2940

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gtccggtggg tatcgttgca aatcagccga tgcagtttgc gggttgcctg gatattaccg 3000

cctctgaaaa agcggcccgc tttgtgcgta cctgtgatgc gttcaacgtg ccggttctga 3060

cgtttgtgga tgttccgggc ttcctgccgg gtgttgatca ggaacatgat ggcattatcc 3120

gccgtggtgc gaaactgatt tttgcgtatg ccgaagcaac cgtgccgctg attaccgtta 3180

tcacgcgcaa agcattcggc ggtgcgtacg atgtgatggg cagcaaacat ctgggtgccg 3240

atctgaacct ggcatggccg accgcacaga tcgcagtgat gggcgcgcag ggtgccgtta 3300

atattctgca ccgccgtacc atcgcagatg caggtgatga tgcagaagcg acgcgcgcac 3360

gtctgattca ggaatatgaa gatgcgctgc tgaacccgta taccgcagcg gaacgtggtt 3420

acgtggatgc ggttattatg ccgagcgata cccgccgtca tatcgtgcgt ggtctgcgtc 3480

agctgcgtac gaaacgtgaa tctctgccgc cgaaaaaaca cggtaatatt ccgctgtaa 3539

<210> SEQ ID NO 16
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 16

aaactgcaga ggaggacagc tatgtctttt agcgaatttt atcag 45

<210> SEQ ID NO 17
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 17

aaaggatccc tattcttcga tcgcctggcg aatttg 36

<210> SEQ ID NO 18
<211> LENGTH: 383
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium bovis

<400> SEQUENCE: 18

Leu Val Glu Gly Leu Arg Glu Val Ala Asp Gly Asp Ala Leu Tyr Asp
1 5 10 15

Ala Ala Val Gly His Gly Asp Arg Gly Pro Val Trp Val Phe Ser Gly
20 25 30

Gln Gly Ser Gln Trp Ala Ala Met Gly Thr Gln Leu Leu Ala Ser Glu
35 40 45

Pro Val Phe Ala Ala Thr Ile Ala Lys Leu Glu Pro Val Ile Ala Ala
50 55 60

Glu Ser Gly Phe Ser Val Thr Glu Ala Ile Thr Ala Gln Gln Thr Val
65 70 75 80

Thr Gly Ile Asp Lys Val Gln Pro Ala Val Phe Ala Val Gln Val Ala
85 90 95

Leu Ala Ala Thr Met Glu Gln Thr Tyr Gly Val Arg Pro Gly Ala Val
100 105 110

Val Gly His Ser Met Gly Glu Ser Ala Ala Ala Val Val Ala Gly Ala
115 120 125

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Leu Ser Leu Glu Asp Ala Ala Arg Val Ile Cys Arg Arg Ser Lys Leu
    130                 135                 140

Met Thr Arg Ile Ala Gly Ala Gly Ala Met Gly Ser Val Glu Leu Pro
145                 150                 155                 160

Ala Lys Gln Val Asn Ser Glu Leu Met Ala Arg Gly Ile Asp Asp Val
                165                 170                 175

Val Val Ser Val Val Ala Ser Pro Gln Ser Thr Val Ile Gly Gly Thr
            180                 185                 190

Ser Asp Thr Val Arg Asp Leu Ile Ala Arg Trp Glu Gln Arg Asp Val
        195                 200                 205

Met Ala Arg Glu Val Ala Val Asp Val Ala Ser His Ser Pro Gln Val
    210                 215                 220

Asp Pro Ile Leu Asp Asp Leu Ala Ala Ala Leu Ala Asp Ile Ala Pro
225                 230                 235                 240

Met Thr Pro Lys Val Pro Tyr Tyr Ser Ala Thr Leu Phe Asp Pro Arg
                245                 250                 255

Glu Gln Pro Val Cys Asp Gly Ala Tyr Trp Val Asp Asn Leu Arg Asn
            260                 265                 270

Thr Val Gln Phe Ala Ala Ala Val Gln Ala Ala Met Glu Asp Gly Tyr
        275                 280                 285

Arg Val Phe Ala Glu Leu Ser Pro His Pro Leu Leu Thr His Ala Val
    290                 295                 300

Glu Gln Thr Gly Arg Ser Leu Asp Met Ser Val Ala Ala Leu Ala Gly
305                 310                 315                 320

Met Arg Arg Glu Gln Pro Leu Pro His Gly Leu Arg Gly Leu Leu Thr
                325                 330                 335

Glu Leu His Arg Ala Gly Ala Ala Leu Asp Tyr Ser Ala Leu Tyr Pro
            340                 345                 350

Ala Gly Arg Leu Val Asp Ala Pro Leu Pro Ala Trp Thr His Ala Arg
        355                 360                 365

Leu Phe Ile Asp Asp Asp Gly Gln Glu Gln Arg Ala Gln Gly Ala
    370                 375                 380


<210> SEQ ID NO 19
<211> LENGTH: 2111
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium bovis
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: GenBank / YP_97046
<309> DATABASE ENTRY DATE: 2010-12-14
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(2111)

<400> SEQUENCE: 19

Met Glu Ser Arg Val Thr Pro Val Ala Val Ile Gly Met Gly Cys Arg
1               5                   10                  15

Leu Pro Gly Gly Ile Asn Ser Pro Asp Lys Leu Trp Glu Ser Leu Leu
            20                  25                  30

Arg Gly Asp Asp Leu Val Thr Glu Ile Pro Pro Asp Arg Trp Asp Ala
        35                  40                  45

Asp Asp Tyr Tyr Asp Pro Glu Pro Gly Val Pro Gly Arg Ser Val Ser
    50                  55                  60

Arg Trp Gly Gly Phe Leu Asp Asp Val Ala Gly Phe Asp Ala Glu Phe
65                  70                  75                  80

Phe Gly Ile Ser Glu Arg Glu Ala Thr Ser Ile Asp Pro Gln Gln Arg
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               85                  90                  95

Leu Leu Leu Glu Thr Ser Trp Glu Ala Ile Glu His Ala Gly Leu Asp
            100                 105                 110

Pro Ala Ser Leu Ala Gly Ser Ser Thr Ala Val Phe Thr Gly Leu Thr
        115                 120                 125

His Glu Asp Tyr Leu Val Leu Thr Thr Thr Ala Gly Gly Leu Ala Ser
    130                 135                 140

Pro Tyr Val Val Thr Gly Leu Asn Asn Ser Val Ala Ser Gly Arg Ile
145                 150                 155                 160

Ala His Thr Leu Gly Leu His Gly Pro Ala Met Thr Phe Asp Thr Ala
                165                 170                 175

Cys Ser Ser Gly Leu Met Ala Val His Leu Ala Cys Arg Ser Leu His
            180                 185                 190

Asp Gly Glu Ala Asp Leu Ala Leu Ala Gly Gly Cys Ala Val Leu Leu
        195                 200                 205

Glu Pro His Ala Cys Val Ala Ala Ser Ala Gln Gly Met Leu Ser Ser
    210                 215                 220

Thr Gly Arg Cys His Ser Phe Asp Ala Asp Ala Asp Gly Phe Val Arg
225                 230                 235                 240

Ser Glu Gly Cys Ala Met Val Leu Leu Lys Arg Leu Pro Asp Ala Leu
                245                 250                 255

Arg Asp Gly Asn Arg Ile Phe Ala Val Val Arg Gly Thr Ala Thr Asn
            260                 265                 270

Gln Asp Gly Arg Thr Glu Thr Leu Thr Met Pro Ser Glu Asp Ala Gln
        275                 280                 285

Val Ala Val Tyr Arg Ala Ala Leu Ala Ala Ala Gly Val Gln Pro Glu
    290                 295                 300

Thr Val Gly Val Val Glu Ala His Gly Thr Gly Thr Pro Ile Gly Asp
305                 310                 315                 320

Pro Ile Glu Tyr Arg Ser Leu Ala Arg Val Tyr Gly Ala Gly Thr Pro
                325                 330                 335

Cys Ala Leu Gly Ser Ala Lys Ser Asn Met Gly His Ser Thr Ala Ser
            340                 345                 350

Ala Gly Thr Val Gly Leu Ile Lys Ala Ile Leu Ser Leu Arg His Gly
        355                 360                 365

Val Val Pro Pro Leu Leu His Phe Asn Arg Leu Pro Asp Glu Leu Ser
    370                 375                 380

Asp Val Glu Thr Gly Leu Phe Val Pro Gln Ala Val Thr Pro Trp Pro
385                 390                 395                 400

Asn Gly Asn Asp His Thr Pro Lys Arg Val Ala Val Ser Ser Phe Gly
                405                 410                 415

Met Ser Gly Thr Asn Val His Ala Ile Val Glu Glu Ala Pro Ala Glu
            420                 425                 430

Ala Ser Ala Pro Glu Ser Ser Pro Gly Asp Ala Glu Val Gly Pro Arg
        435                 440                 445

Leu Phe Met Leu Ser Ser Thr Ser Ser Asp Ala Leu Arg Gln Thr Ala
    450                 455                 460

Arg Gln Leu Ala Thr Trp Val Glu Glu His Gln Asp Cys Val Ala Ala
465                 470                 475                 480

Ser Asp Leu Ala Tyr Thr Leu Ala Arg Gly Arg Ala His Arg Pro Val
                485                 490                 495
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Arg Thr Ala Val Val Ala Ala Asn Leu Pro Glu Leu Val Glu Gly Leu
            500                 505                 510

Arg Glu Val Ala Asp Gly Asp Ala Leu Tyr Asp Ala Ala Val Gly His
        515                 520                 525

Gly Asp Arg Gly Pro Val Trp Val Phe Ser Gly Gln Gly Ser Gln Trp
    530                 535                 540

Ala Ala Met Gly Thr Gln Leu Leu Ala Ser Glu Pro Val Phe Ala Ala
545                 550                 555                 560

Thr Ile Ala Lys Leu Glu Pro Val Ile Ala Ala Glu Ser Gly Phe Ser
                565                 570                 575

Val Thr Glu Ala Ile Thr Ala Gln Gln Thr Val Thr Gly Ile Asp Lys
            580                 585                 590

Val Gln Pro Ala Val Phe Ala Val Gln Val Ala Leu Ala Ala Thr Met
        595                 600                 605

Glu Gln Thr Tyr Gly Val Arg Pro Gly Ala Val Val Gly His Ser Met
    610                 615                 620

Gly Glu Ser Ala Ala Ala Val Val Ala Gly Ala Leu Ser Leu Glu Asp
625                 630                 635                 640

Ala Ala Arg Val Ile Cys Arg Arg Ser Lys Leu Met Thr Arg Ile Ala
                645                 650                 655

Gly Ala Gly Ala Met Gly Ser Val Glu Leu Pro Ala Lys Gln Val Asn
            660                 665                 670

Ser Glu Leu Met Ala Arg Gly Ile Asp Asp Val Val Val Ser Val Val
        675                 680                 685

Ala Ser Pro Gln Ser Thr Val Ile Gly Gly Thr Ser Asp Thr Val Arg
    690                 695                 700

Asp Leu Ile Ala Arg Trp Glu Gln Arg Asp Val Met Ala Arg Glu Val
705                 710                 715                 720

Ala Val Asp Val Ala Ser His Ser Pro Gln Val Asp Pro Ile Leu Asp
                725                 730                 735

Asp Leu Ala Ala Ala Leu Ala Asp Ile Ala Pro Met Thr Pro Lys Val
            740                 745                 750

Pro Tyr Tyr Ser Ala Thr Leu Phe Asp Pro Arg Glu Gln Pro Val Cys
        755                 760                 765

Asp Gly Ala Tyr Trp Val Asp Asn Leu Arg Asn Thr Val Gln Phe Ala
    770                 775                 780

Ala Ala Val Gln Ala Ala Met Glu Asp Gly Tyr Arg Val Phe Ala Glu
785                 790                 795                 800

Leu Ser Pro His Pro Leu Leu Thr His Ala Val Glu Gln Thr Gly Arg
                805                 810                 815

Ser Leu Asp Met Ser Val Ala Ala Leu Ala Gly Met Arg Arg Glu Gln
            820                 825                 830

Pro Leu Pro His Gly Leu Arg Gly Leu Leu Thr Glu Leu His Arg Ala
        835                 840                 845

Gly Ala Ala Leu Asp Tyr Ser Ala Leu Tyr Pro Ala Gly Arg Leu Val
    850                 855                 860

Asp Ala Pro Leu Pro Ala Trp Thr His Ala Arg Leu Phe Ile Asp Asp
865                 870                 875                 880

Asp Gly Gln Glu Gln Arg Ala Gln Gly Ala Cys Thr Ile Thr Val His
                885                 890                 895
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Pro Leu Leu Gly Ser His Val Arg Leu Thr Glu Glu Pro Glu Arg His
            900                 905                 910

Val Trp Gln Gly Asp Val Gly Thr Ser Val Leu Ser Trp Leu Ser Asp
        915                 920                 925

His Gln Val His Asn Val Ala Ala Leu Pro Gly Ala Ala Tyr Cys Glu
    930                 935                 940

Met Ala Leu Ala Ala Ala Ala Glu Val Phe Gly Glu Ala Ala Glu Val
945                 950                 955                 960

Arg Asp Ile Thr Phe Glu Gln Met Leu Leu Leu Asp Glu Gln Thr Pro
                965                 970                 975

Ile Asp Ala Val Ala Ser Ile Asp Ala Pro Gly Val Val Asn Phe Thr
            980                 985                 990

Val Glu Thr Asn Arg Asp Gly Glu  Thr Thr Arg His Ala  Thr Ala Ala
        995                 1000                1005

Leu Arg  Ala Ala Glu Asp Asp  Cys Pro Pro Pro Gly  Tyr Asp Ile
    1010                1015                1020

Thr Ala  Leu Leu Gln Ala His  Pro His Ala Val Asn  Gly Thr Ala
    1025                1030                1035

Met Arg  Glu Ser Phe Ala Glu  Arg Gly Val Thr Leu  Gly Ala Ala
    1040                1045                1050

Phe Gly  Gly Leu Thr Thr Ala  His Thr Ala Glu Ala  Gly Ala Ala
    1055                1060                1065

Thr Val  Leu Ala Glu Val Ala  Leu Pro Ala Ser Ile  Arg Phe Gln
    1070                1075                1080

Gln Gly  Ala Tyr Arg Ile His  Pro Ala Leu Leu Asp  Ala Cys Phe
    1085                1090                1095

Gln Ser  Val Gly Ala Gly Val  Gln Ala Gly Thr Ala  Thr Gly Gly
    1100                1105                1110

Leu Leu  Leu Pro Leu Gly Val  Arg Ser Leu Arg Ala  Tyr Gly Pro
    1115                1120                1125

Thr Arg  Asn Ala Arg Tyr Cys  Tyr Thr Arg Leu Thr  Lys Ala Phe
    1130                1135                1140

Asn Asp  Gly Thr Arg Gly Gly  Glu Ala Asp Leu Asp  Val Leu Asp
    1145                1150                1155

Glu His  Gly Thr Val Leu Leu  Ala Val Arg Gly Leu  Arg Met Gly
    1160                1165                1170

Thr Gly  Thr Ser Glu Arg Asp  Glu Arg Asp Arg Leu  Val Ser Glu
    1175                1180                1185

Arg Leu  Leu Thr Leu Gly Trp  Gln Gln Arg Ala Leu  Pro Glu Val
    1190                1195                1200

Gly Asp  Gly Glu Ala Gly Ser  Trp Leu Leu Ile Asp  Thr Ser Asn
    1205                1210                1215

Ala Val  Asp Thr Pro Asp Met  Leu Ala Ser Thr Leu  Thr Asp Ala
    1220                1225                1230

Leu Lys  Ser His Gly Pro Gln  Gly Thr Glu Cys Ala  Ser Leu Ser
    1235                1240                1245

Trp Ser  Val Gln Asp Thr Pro  Pro Asn Asp Gln Ala  Gly Leu Glu
    1250                1255                1260

Lys Leu  Gly Ser Gln Leu Arg  Gly Arg Asp Gly Val  Val Ile Val
    1265                1270                1275

Tyr Gly  Pro Arg Val Gly Asp  Pro Asp Glu His Ser  Leu Leu Ala
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    1280                1285                1290

Gly Arg  Glu Gln Val Arg His  Leu Val Arg Ile Thr  Arg Glu Leu
    1295                1300                1305

Ala Glu  Phe Glu Gly Glu Leu  Pro Arg Leu Phe Val  Val Thr Arg
    1310                1315                1320

Gln Ala  Gln Ile Val Lys Pro  His Asp Ser Gly Glu  Arg Ala Asn
    1325                1330                1335

Leu Glu  Gln Ala Gly Leu Arg  Gly Leu Leu Arg Val  Ile Ser Ser
    1340                1345                1350

Glu His  Pro Met Leu Arg Thr  Thr Leu Ile Asp Val  Asp Glu His
    1355                1360                1365

Thr Asp  Val Glu Arg Val Ala  Gln Gln Leu Leu Ser  Gly Ser Glu
    1370                1375                1380

Glu Asp  Glu Thr Ala Trp Arg  Asn Gly Asp Trp Tyr  Val Ala Arg
    1385                1390                1395

Leu Thr  Pro Ser Pro Leu Gly  His Glu Glu Arg Arg  Thr Ala Val
    1400                1405                1410

Leu Asp  Pro Asp His Asp Gly  Met Arg Val Gln Val  Arg Arg Pro
    1415                1420                1425

Gly Asp  Leu Gln Thr Leu Glu  Phe Val Ala Ser Asp  Arg Val Pro
    1430                1435                1440

Pro Gly  Pro Gly Gln Ile Glu  Val Ala Val Ser Met  Ser Ser Ile
    1445                1450                1455

Asn Phe  Ala Asp Val Leu Ile  Ala Phe Gly Arg Phe  Pro Ile Ile
    1460                1465                1470

Asp Asp  Arg Glu Pro Gln Leu  Gly Met Asp Phe Val  Gly Val Val
    1475                1480                1485

Thr Ala  Val Gly Glu Gly Val  Thr Gly His Gln Val  Gly Asp Arg
    1490                1495                1500

Val Gly  Gly Phe Ser Glu Gly  Gly Cys Trp Arg Thr  Phe Leu Thr
    1505                1510                1515

Cys Asp  Ala Asn Leu Ala Val  Thr Leu Pro Pro Gly  Leu Thr Asp
    1520                1525                1530

Glu Gln  Ala Ile Thr Ala Ala  Thr Ala His Ala Thr  Ala Trp Tyr
    1535                1540                1545

Gly Leu  Asn Asp Leu Ala Gln  Ile Lys Ala Gly Asp  Lys Val Leu
    1550                1555                1560

Ile His  Ser Ala Thr Gly Gly  Val Gly Gln Ala Ala  Ile Ser Ile
    1565                1570                1575

Ala Arg  Ala Lys Gly Ala Glu  Ile Phe Ala Thr Ala  Gly Asn Pro
    1580                1585                1590

Ala Lys  Arg Ala Met Leu Arg  Asp Met Gly Val Glu  His Val Tyr
    1595                1600                1605

Asp Ser  Arg Ser Val Glu Phe  Ala Glu Gln Ile Arg  Arg Asp Thr
    1610                1615                1620

Asp Gly  Tyr Gly Val Asp Ile  Val Leu Asn Ser Leu  Thr Gly Ala
    1625                1630                1635

Ala Gln  Arg Ala Gly Leu Glu  Leu Leu Ala Phe Gly  Gly Arg Phe
    1640                1645                1650

Val Glu  Ile Gly Lys Ala Asp  Val Tyr Gly Asn Thr  Arg Leu Gly
    1655                1660                1665
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Leu Phe  Pro Phe Arg Arg Gly  Leu Thr Phe Tyr Tyr  Leu Asp Leu
    1670                1675                1680

Ala Leu  Met Ser Val Thr Gln  Pro Asp Arg Val Arg  Glu Leu Leu
    1685                1690                1695

Ala Thr  Val Phe Lys Leu Thr  Ala Asp Gly Val Leu  Thr Ala Pro
    1700                1705                1710

Gln Cys  Thr His Tyr Pro Leu  Ala Glu Ala Ala Asp  Ala Ile Arg
    1715                1720                1725

Ala Met  Ser Asn Ala Glu His  Thr Gly Lys Leu Val  Leu Asp Val
    1730                1735                1740

Pro Arg  Ser Gly Arg Arg Ser  Val Ala Val Thr Pro  Glu Gln Ala
    1745                1750                1755

Pro Leu  Tyr Arg Arg Asp Gly  Ser Tyr Ile Ile Thr  Gly Gly Leu
    1760                1765                1770

Gly Gly  Leu Gly Leu Phe Phe  Ala Ser Lys Leu Ala  Ala Ala Gly
    1775                1780                1785

Cys Gly  Arg Ile Val Leu Thr  Ala Arg Ser Gln Pro  Asn Pro Lys
    1790                1795                1800

Ala Arg  Gln Thr Ile Glu Gly  Leu Arg Ala Ala Gly  Ala Asp Ile
    1805                1810                1815

Val Val  Glu Cys Gly Asn Ile  Ala Glu Pro Asp Thr  Ala Asp Arg
    1820                1825                1830

Leu Val  Ser Ala Ala Thr Ala  Thr Gly Leu Pro Leu  Arg Gly Val
    1835                1840                1845

Leu His  Ser Ala Ala Val Val  Glu Asp Ala Thr Leu  Thr Asn Ile
    1850                1855                1860

Thr Asp  Glu Leu Ile Asp Arg  Asp Trp Ser Pro Lys  Val Phe Gly
    1865                1870                1875

Ser Trp  Asn Leu His Arg Ala  Thr Leu Gly Gln Pro  Leu Asp Trp
    1880                1885                1890

Phe Cys  Leu Phe Ser Ser Gly  Ala Ala Leu Leu Gly  Ser Pro Gly
    1895                1900                1905

Gln Gly  Ala Tyr Ala Ala Ala  Asn Ser Trp Val Asp  Val Phe Ala
    1910                1915                1920

His Trp  Arg Arg Ala Gln Gly  Leu Pro Val Ser Ala  Ile Ala Trp
    1925                1930                1935

Gly Ala  Trp Gly Glu Val Gly  Arg Ala Thr Phe Leu  Ala Glu Gly
    1940                1945                1950

Gly Glu  Ile Met Ile Thr Pro  Glu Glu Gly Ala Tyr  Ala Phe Glu
    1955                1960                1965

Thr Leu  Val Arg His Asp Arg  Ala Tyr Ser Gly Tyr  Ile Pro Ile
    1970                1975                1980

Leu Gly  Ala Pro Trp Leu Ala  Asp Leu Val Arg Arg  Ser Pro Trp
    1985                1990                1995

Gly Glu  Met Phe Ala Ser Thr  Gly Gln Arg Ser Arg  Gly Pro Ser
    2000                2005                2010

Lys Phe  Arg Met Glu Leu Leu  Ser Leu Pro Gln Asp  Glu Trp Ala
    2015                2020                2025

Gly Arg  Leu Arg Arg Leu Leu  Val Glu Gln Ala Ser  Val Ile Leu
    2030                2035                2040
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Arg Arg  Thr Ile Asp Ala Asp  Arg Ser Phe Ile Glu  Tyr Gly Leu
    2045                2050                2055

Asp Ser  Leu Gly Met Leu Glu  Met Arg Thr His Val  Glu Thr Glu
    2060                2065                2070

Thr Gly  Ile Arg Leu Thr Pro  Lys Val Ile Ala Thr  Asn Asn Thr
    2075                2080                2085

Ala Arg  Ala Leu Ala Gln Tyr  Leu Ala Asp Thr Leu  Ala Glu Glu
    2090                2095                2100

Gln Ala  Ala Ala Pro Ala Ala  Ser
    2105                2110
```

<210> SEQ ID NO 20
<211> LENGTH: 1149
<212> TYPE: DNA
<213> ORGANISM: Mycobacterium bovis

<400> SEQUENCE: 20

```
ctggtggaag gcctgcgtga agttgccgat ggtgatgcac tgtatgatgc agcagtgggt     60

catggcgatc gtggtccggt ttgggtgttt agcggccagg gttctcagtg ggcagcgatg    120

ggcacccagc tgctggcaag cgaaccggtt tttgccgcaa cgattgcaaa actggaaccg    180

gtgatcgcgg ccgaaagtgg cttcagcgtt accgaagcaa ttacggcgca gcagaccgtg    240

acgggtatcg ataaagtgca gccggccgtt ttcgcagttc aggtggcgct ggcagcgacg    300

atggaacaga cgtacggcgt tcgtccgggt gcagtggttg gtcacagtat gggtgaaagc    360

gccgcagcgg tggttgcagg cgccctgagt ctggaagatg ccgcacgtgt gatttgccgt    420

cgcagcaaac tgatgacccg tatcgcaggt gcaggtgcga tgggcagcgt ggaactgccg    480

gcaaaacagg ttaactctga actgatggcg cgcggtattg atgatgtggt tgtgtctgtt    540

gtggcgtctc cgcagagtac cgtgattggc ggcaccagtg atacggttcg tgatctgatc    600

gcgcgttggg aacagcgcga tgtgatggcg cgcgaagttg ccgtggatgt tgcaagccat    660

tctccgcagg ttgatccgat tctggatgat ctggcggcgg cactggcaga tattgcaccg    720

atgaccccga aagtgccgta ttacagcgcg acgctgtttg atccgcgtga acagccggtg    780

tgtgatggcg cctattgggt tgataacctg cgcaataccg tgcagtttgc ggcggcagtt    840

caggcggcga tggaagatgg ttaccgtgtg ttcgcggaac tgtctccgca tccgctgctg    900

acccacgcag tggaacagac gggtcgctct ctggatatga gtgttgcagc actggccggt    960

atgcgtcgcg aacagccgct gccgcatggc ctgcgtggtc tgctgaccga actgcaccgt   1020

gcaggtgcag cactggatta tagcgcactg tacccggcag gtcgtctggt ggatgcaccg   1080

ctgccggcat ggacgcacgc acgtctgttc atcgatgatg atggccagga acagcgcgca   1140

cagggtgcg                                                           1149
```

<210> SEQ ID NO 21
<211> LENGTH: 1149
<212> TYPE: DNA
<213> ORGANISM: Mycobacterium bovis

<400> SEQUENCE: 21

```
ctcgtcgagg gtttgcgcga ggtggccgac ggtgacgccc tctatgacgc ggcggtggga     60

cacggtgatc gaggaccggt ctgggtcttc tccgggcaag ggtcgcagtg ggcggcgatg    120

ggcacgcaat tgctcgccag cgaaccagtg ttcgcggcca ccatcgccaa gctggagccg    180
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gtgatcgccg cagaatcggg attctcggtg accgaggcga taacggcgca gcagaccgtg 240

accggaatcg acaaagtgca gccggcagtg ttcgccgttc aggtcgcgtt ggccgccacc 300

atggagcaaa cctacggagt gcggccgggc gcggtcgtcg gacactcgat gggtgagtcg 360

gccgcggccg tcgtcgcggg ggcactgtcg ctcgaggacg cggcgcgcgt catttgccgc 420

cgctcgaagc tgatgacccg catagccggt gctggtgcca tgggctcggt ggaattgccc 480

gccaagcaag tgaattcgga gctgatggca cgcggaatcg acgatgttgt ggtctcggtg 540

gtggcgtccc cgcaatccac ggtgatcggc ggtacgagcg acaccgttcg tgacctcatc 600

gcccgttggg agcagcggga cgtgatggcg cgcgaggtgg ccgtcgacgt ggcgtcgcac 660

tcgcctcaag tcgatccgat actcgacgat ttggccgcgg cgctggcgga cattgctccg 720

atgacgccca aggtgccgta ctactcggcg accctgttcg acccgcgcga gcagccggtg 780

tgcgatggcg cttactgggt ggacaatctg cgcaacacgg tgcagttcgc cgcggcggtg 840

caggctgcga tggaggacgg ctaccgggtc ttcgcggagc tgtcgcccca cccgctgctt 900

acccacgccg tcgaacagac gggccgaagc ctcgacatgt cggtcgccgc cctggccggc 960

atgcggcgag agcagcctct gccgcatggt ctgcgcggct tgctgacgga gctgcaccgc 1020

gcgggcgccg ctttggacta ttcggcgctg tatcccgctg ggcggctggt ggatgcgccg 1080

ctgccggcgt ggacccacgc ccgcctattc atcgacgatg atgggcaaga acagcgggca 1140

caaggtgcc 1149

<210> SEQ ID NO 22
<211> LENGTH: 628
<212> TYPE: PRT
<213> ORGANISM: Salmonella enterica
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: GenBank / AAC44817
<309> DATABASE ENTRY DATE: 1999-08-05
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(628)

<400> SEQUENCE: 22

Met Ser Phe Ser Glu Phe Tyr Gln Arg Ser Ile Asn Glu Pro Glu Ala
1 5 10 15

Phe Trp Ala Glu Gln Ala Arg Arg Ile Asp Trp Arg Gln Pro Phe Thr
20 25 30

Gln Thr Leu Asp His Ser Arg Pro Pro Phe Ala Arg Trp Phe Cys Gly
35 40 45

Gly Thr Thr Asn Leu Cys His Asn Ala Val Asp Arg Trp Arg Asp Lys
50 55 60

Gln Pro Glu Ala Leu Ala Leu Ile Ala Val Ser Ser Glu Thr Asp Glu
65 70 75 80

Glu Arg Thr Phe Thr Phe Ser Gln Leu His Asp Glu Val Asn Ile Val
85 90 95

Ala Ala Met Leu Leu Ser Leu Gly Val Gln Arg Gly Asp Arg Val Leu
100 105 110

Val Tyr Met Pro Met Ile Ala Glu Ala Gln Ile Thr Leu Leu Ala Cys
115 120 125

Ala Arg Ile Gly Ala Ile His Ser Val Val Phe Gly Gly Phe Ala Ser
130 135 140

His Ser Val Ala Ala Arg Ile Asp Asp Ala Arg Pro Ala Leu Ile Val
145 150 155 160

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Ser Ala Asp Ala Gly Ala Arg Gly Gly Lys Ile Leu Pro Tyr Lys Lys
                165                 170                 175

Leu Leu Asp Asp Ala Ile Ala Gln Ala Gln His Gln Pro Lys His Val
            180                 185                 190

Leu Leu Val Asp Arg Gly Leu Ala Lys Met Ala Trp Val Asp Gly Arg
        195                 200                 205

Asp Leu Asp Phe Ala Thr Leu Arg Gln Gln His Leu Gly Ala Ser Val
    210                 215                 220

Pro Val Ala Trp Leu Glu Ser Asn Glu Thr Ser Cys Ile Leu Tyr Thr
225                 230                 235                 240

Ser Gly Thr Thr Gly Lys Pro Lys Gly Val Gln Arg Asp Val Gly Gly
                245                 250                 255

Tyr Ala Val Ala Leu Ala Thr Ser Met Asp Thr Ile Phe Gly Gly Lys
            260                 265                 270

Ala Gly Gly Val Phe Phe Cys Ala Ser Asp Ile Gly Trp Val Val Gly
        275                 280                 285

His Ser Tyr Ile Val Tyr Ala Pro Leu Leu Ala Gly Met Ala Thr Ile
    290                 295                 300

Val Tyr Glu Gly Leu Pro Thr Tyr Pro Asp Cys Gly Val Trp Trp Lys
305                 310                 315                 320

Ile Val Glu Lys Tyr Gln Val Asn Arg Met Phe Ser Ala Pro Thr Ala
                325                 330                 335

Ile Arg Val Leu Lys Lys Phe Pro Thr Ala Gln Ile Arg Asn His Asp
            340                 345                 350

Leu Ser Ser Leu Glu Ala Leu Tyr Leu Ala Gly Glu Pro Leu Asp Glu
        355                 360                 365

Pro Thr Ala Ser Trp Val Thr Glu Thr Leu Gly Val Pro Val Ile Asp
    370                 375                 380

Asn Tyr Trp Gln Thr Glu Ser Gly Trp Pro Ile Met Ala Leu Ala Arg
385                 390                 395                 400

Ala Leu Asp Asp Arg Pro Ser Arg Leu Gly Ser Pro Gly Val Pro Met
                405                 410                 415

Tyr Gly Tyr Asn Val Gln Leu Leu Asn Glu Val Thr Gly Glu Pro Cys
            420                 425                 430

Gly Ile Asn Glu Lys Gly Met Leu Val Ile Glu Gly Pro Leu Pro Pro
        435                 440                 445

Gly Cys Ile Gln Thr Ile Trp Gly Asp Asp Ala Arg Phe Val Lys Thr
    450                 455                 460

Tyr Trp Ser Leu Phe Asn Arg Gln Val Tyr Ala Thr Phe Asp Trp Gly
465                 470                 475                 480

Ile Arg Asp Ala Glu Gly Tyr Tyr Phe Ile Leu Gly Arg Thr Asp Asp
                485                 490                 495

Val Ile Asn Ile Ala Gly His Arg Leu Gly Thr Arg Glu Ile Glu Glu
            500                 505                 510

Ser Ile Ser Ser Tyr Pro Asn Val Ala Glu Val Ala Val Val Gly Ile
        515                 520                 525

Lys Asp Ala Leu Lys Gly Gln Val Ala Val Ala Phe Val Ile Pro Lys
    530                 535                 540

Gln Ser Asp Thr Leu Ala Asp Arg Glu Ala Ala Arg Asp Glu Glu Asn
545                 550                 555                 560
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```
Ala Ile Met Ala Leu Val Asp Asn Gln Ile Gly His Phe Gly Arg Pro
               565                 570                 575

Ala His Val Trp Phe Val Ser Gln Leu Pro Lys Thr Arg Ser Gly Lys
           580                 585                 590

Met Leu Arg Arg Thr Ile Gln Ala Ile Cys Glu Gly Arg Asp Pro Gly
       595                 600                 605

Asp Leu Thr Thr Ile Asp Asp Pro Ala Ser Leu Gln Gln Ile Arg Gln
   610                 615                 620

Ala Ile Glu Glu
625
```

<210> SEQ ID NO 23
<211> LENGTH: 1884
<212> TYPE: DNA
<213> ORGANISM: Salmonella enterica

<400> SEQUENCE: 23

```
atgtctttta gcgaatttta tcagcgttcc attaacgaac cggaggcgtt ctgggccgag     60

caggcccggc gtatcgactg gcgacagccg tttacgcaga cgctggatca tagccgtcca    120

ccgtttgccc gctggttttg cggcggcacc actaacttat gtcataacgc cgtcgaccgc    180

tggcgggata aacagccgga ggcgctggcg ctgattgccg tctcatcaga gaccgatgaa    240

gagcgcacat ttaccttcag ccagttgcat gatgaagtca acattgtggc cgccatgttg    300

ctgtcgctgg gcgtgcagcg tggcgatcgc gtattggtct atatgccgat gattgccgaa    360

gcgcagataa ccctgctggc ctgcgcgcgc attggcgcga tccattcggt ggtctttggc    420

ggttttgcct cgcacagcgt ggcggcgcgc attgacgatg ccagaccggc gctgattgtg    480

tcggcggatg ccggagcgcg gggcggtaaa atcctgccgt ataaaaagct gctcgatgac    540

gctattgcgc aggcgcagca tcagccgaaa cacgttctgc tggtggacag agggctggcg    600

aaaatggcat gggtggatgg gcgcgatctg gattttgcca cgttgcgcca gcagcatctc    660

ggcgcgagcg tgccggtggc gtggctggaa tccaacgaaa cctcgtgcat tctttacacc    720

tccggcacta ccggcaaacc gaaaggcgtc cagcgcgacg tcggcggtta tgcggtggcg    780

ctggcaacct cgatggacac catttttggc ggcaaggcgg gcggcgtatt cttttgcgca    840

tcggatatcg gctgggtcgt cggccactcc tatatcgttt acgcgccgtt gctggcaggc    900

atggcgacta ttgtttacga aggactgccg acgtacccgg actgcggggt ctggtggaaa    960

attgtcgaga aataccaggt taaccggatg ttttccgccc cgaccgcgat tcgcgtgctg   1020

aaaaaattcc cgacggcgca aatccgcaat cacgatctct cctcgctgga ggcgctttat   1080

ctggccggtg agccgctgga cgagccgacg gccagttggg taacggagac gctgggcgta   1140

ccggtcatcg acaattattg gcagacggag tccggctggc cgatcatggc gctggcccgc   1200

gcgctggacg acaggccgtc gcgtctggga agtcccggcg tgccgatgta cggttataac   1260

gtccagctac tcaatgaagt caccggcgaa ccttgcggca taaatgaaaa ggggatgctg   1320

gtgatcgaag ggccgctgcc gccgggctgt attcagacta tttggggcga cgatgcgcgt   1380

tttgtgaaga cttactggtc gctgtttaac cgtcaggttt atgccacttt cgactgggga   1440

atccgcgacg ccgaggggta ttactttatt ctgggccgta ccgatgatgt gattaatatt   1500

gcgggtcatc ggctggggac gcgagaaata gaagaaagta tctccagcta cccgaacgta   1560

gcggaagtgg cggtagtggg gataaaagac gctctgaaag ggcaggtagc ggtggcgttt   1620
```

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gtcattccga agcagagcga tacgctggcg gatcgcgagg cggcgcgcga cgaggaaaac 1680

gcgattatgg cgctggtgga caaccagatc ggtcactttg gtcgtccggc gcatgtctgg 1740

tttgtttcgc agctccccaa aacgcgttcc ggaaagatgc ttcgccgcac gatccaggcg 1800

atctgcgaag gccgcgatcc gggcgatctg acaaccattg acgatcccgc gtcgttgcag 1860

caaattcgcc aggcgatcga agaa 1884

<210> SEQ ID NO 24
<211> LENGTH: 616
<212> TYPE: PRT
<213> ORGANISM: Streptomyces cinnamonensis

<400> SEQUENCE: 24

Met Thr Val Leu Pro Asp Asp Gly Leu Ser Leu Ala Ala Glu Phe Pro
1 5 10 15

Asp Ala Thr His Glu Gln Trp His Arg Leu Val Glu Gly Val Val Arg
20 25 30

Lys Ser Gly Lys Asp Val Ser Gly Thr Ala Ala Glu Glu Ala Leu Ser
35 40 45

Thr Thr Leu Glu Asp Gly Leu Thr Thr Arg Pro Leu Tyr Thr Ala Arg
50 55 60

Asp Ala Ala Pro Asp Ala Gly Phe Pro Gly Phe Ala Pro Phe Val Arg
65 70 75 80

Gly Ser Val Pro Glu Gly Asn Thr Pro Gly Gly Trp Asp Val Arg Gln
85 90 95

Arg Tyr Ala Ser Ala Asp Pro Ala Arg Thr Asn Glu Ala Val Leu Thr
100 105 110

Asp Leu Glu Asn Gly Val Thr Ser Leu Trp Leu Thr Leu Gly Ser Ala
115 120 125

Gly Leu Pro Val Thr Gly Leu Glu Arg Ala Leu Asp Gly Val Tyr Leu
130 135 140

Asp Leu Val Pro Val Ala Leu Asp Ala Gly Ser Glu Ala Ala Thr Ala
145 150 155 160

Ala Arg Glu Leu Leu Arg Leu Tyr Glu Ala Ala Gly Val Ala Asp Asp
165 170 175

Ala Val Arg Gly Thr Leu Gly Ala Asp Pro Leu Gly His Glu Ala Arg
180 185 190

Thr Gly Glu Lys Ser Thr Ser Phe Ala Ala Val Ala Glu Leu Ala Arg
195 200 205

Leu Cys Gly Glu Arg Tyr Pro Gly Leu Arg Ala Leu Thr Val Asp Ala
210 215 220

Leu Pro Tyr His Glu Ala Gly Ala Ser Ala Ala Gln Glu Leu Gly Ala
225 230 235 240

Ser Leu Ala Thr Gly Val Glu Tyr Leu Arg Ala Leu His Asp Lys Gly
245 250 255

Leu Gly Val Glu Lys Ala Phe Ala Gln Leu Glu Phe Arg Phe Ala Ala
260 265 270

Thr Ala Asp Gln Phe Leu Thr Ile Ala Lys Leu Arg Ala Ala Arg Arg
275 280 285

Leu Trp Ala Arg Val Ala Glu Val Ser Gly Val Pro Ala Ala Gly Ala
290 295 300

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```
Gln Arg Gln His Ala Val Thr Ser Pro Val Met Met Thr Arg Arg Asp
305                 310                 315                 320

Pro Trp Val Asn Met Leu Arg Thr Thr Val Ala Cys Leu Gly Ala Gly
                325                 330                 335

Val Gly Gly Ala Asp Ala Val Thr Val Leu Pro Phe Asp His Glu Leu
            340                 345                 350

Gly Leu Pro Asp Ala Phe Ala Arg Arg Ile Ala Arg Asn Thr Ser Thr
        355                 360                 365

Ile Leu Leu Glu Glu Ser His Leu Ala Arg Val Ile Asp Pro Ala Gly
    370                 375                 380

Gly Ser Trp Tyr Val Glu Arg Leu Thr Asp Glu Leu Ala His Ala Ala
385                 390                 395                 400

Trp Asp Phe Phe Lys Glu Ile Glu Arg Ala Asp Gly Gln Val Ala Ala
                405                 410                 415

Leu Arg Ser Gly Leu Val Gly Asp Arg Ile Ala Ala Thr Trp Ala Glu
            420                 425                 430

Arg Arg Lys Lys Leu Ala Arg Arg Arg Glu Pro Ile Thr Gly Val Ser
        435                 440                 445

Glu Phe Pro Leu Leu Thr Glu Arg Pro Val Glu Arg Glu Pro Ala Pro
    450                 455                 460

Ala Ala Pro Pro Gly Gly Leu Pro Arg Val Arg Arg Asp Glu Ala Tyr
465                 470                 475                 480

Glu Glu Leu Arg Gly Arg Ser Asp Ala His Leu Glu Ala Thr Gly Ala
                485                 490                 495

Arg Pro Lys Val Phe Ile Ala Ala Leu Gly Pro Ala Ala Ala His Thr
            500                 505                 510

Ala Arg Ala Thr Phe Ala Ala Asn Leu Phe Met Ala Gly Gly Val Glu
        515                 520                 525

Pro Val His Asp Pro Val Ser Val Asp Ala Glu Thr Ala Ala Glu Ala
    530                 535                 540

Phe Ala Ala Ser Gly Ala Thr Val Ala Cys Leu Cys Ser Ser Asp Val
545                 550                 555                 560

Leu Tyr Ala Glu Gln Ala Glu Ala Val Ala Arg Ala Leu Lys Ser Ala
                565                 570                 575

Gly Ala Leu Arg Val Phe Leu Ala Gly Arg Gly Glu Phe Ala Asp Ile
            580                 585                 590

Asp Glu Tyr Val Phe Ala Gly Cys Asp Ala Val Ala Val Leu Thr Ser
        595                 600                 605

Thr Leu Asp Arg Met Gly Val Ala
    610                 615


<210> SEQ ID NO 25
<211> LENGTH: 733
<212> TYPE: PRT
<213> ORGANISM: Streptomyces cinnamonensis

<400> SEQUENCE: 25

Met Arg Ile Pro Glu Phe Asp Asp Ile Glu Leu Gly Ala Gly Gly Gly
1               5                   10                  15

Pro Ser Gly Ser Ala Glu Gln Trp Arg Ala Ala Val Lys Glu Ser Val
            20                  25                  30

Gly Lys Ser Glu Ser Asp Leu Leu Trp Glu Thr Pro Glu Gly Ile Ala
        35                  40                  45
```

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```
Val Lys Pro Leu Tyr Thr Gly Ala Asp Val Glu Gly Leu Asp Phe Leu
    50                  55                  60

Glu Thr Tyr Pro Gly Val Ala Pro Tyr Leu Arg Gly Pro Tyr Pro Thr
65                  70                  75                  80

Met Tyr Val Asn Gln Pro Trp Thr Ile Arg Gln Tyr Ala Gly Phe Ser
                85                  90                  95

Thr Ala Glu Glu Ser Asn Ala Phe Tyr Arg Arg Asn Leu Ala Ala Gly
            100                 105                 110

Gln Lys Gly Leu Ser Val Ala Phe Asp Leu Pro Thr His Arg Gly Tyr
        115                 120                 125

Asp Ser Asp His Pro Arg Val Thr Gly Asp Val Gly Met Ala Gly Val
    130                 135                 140

Ala Ile Asp Ser Ile Tyr Asp Met Arg Gln Leu Phe Asp Gly Ile Pro
145                 150                 155                 160

Leu Asp Lys Met Thr Val Ser Met Thr Met Asn Gly Ala Val Leu Pro
                165                 170                 175

Val Leu Ala Leu Tyr Ile Val Ala Ala Glu Glu Gln Gly Val Pro Pro
            180                 185                 190

Glu Lys Leu Ala Gly Thr Ile Gln Asn Asp Ile Leu Lys Glu Phe Met
        195                 200                 205

Val Arg Asn Thr Tyr Ile Tyr Pro Pro Lys Pro Ser Met Arg Ile Ile
    210                 215                 220

Ser Asp Ile Phe Ala Tyr Thr Ser Gln Lys Met Pro Arg Tyr Asn Ser
225                 230                 235                 240

Ile Ser Ile Ser Gly Tyr His Ile Gln Glu Ala Gly Ala Thr Ala Asp
                245                 250                 255

Leu Glu Leu Ala Tyr Thr Leu Ala Asp Gly Val Glu Tyr Leu Arg Ala
            260                 265                 270

Gly Gln Glu Ala Gly Leu Asp Val Asp Ala Phe Ala Pro Arg Leu Ser
        275                 280                 285

Phe Phe Trp Ala Ile Gly Met Asn Phe Phe Met Glu Val Ala Lys Leu
    290                 295                 300

Arg Ala Ala Arg Leu Leu Trp Ala Lys Leu Val Lys Gln Phe Asp Pro
305                 310                 315                 320

Lys Asn Ala Lys Ser Leu Ser Leu Arg Thr His Ser Gln Thr Ser Gly
                325                 330                 335

Trp Ser Leu Thr Ala Gln Asp Val Phe Asn Asn Val Thr Arg Thr Cys
            340                 345                 350

Val Glu Ala Met Ala Ala Thr Gln Gly His Thr Gln Ser Leu His Thr
        355                 360                 365

Asn Ala Leu Asp Glu Ala Leu Ala Leu Pro Thr Asp Phe Ser Ala Arg
    370                 375                 380

Ile Ala Arg Asn Thr Gln Leu Leu Ile Gln Gln Glu Ser Gly Thr Thr
385                 390                 395                 400

Arg Thr Ile Asp Pro Trp Gly Gly Ser Ala Tyr Val Glu Lys Leu Thr
                405                 410                 415

Tyr Asp Leu Ala Arg Arg Ala Trp Gln His Ile Glu Glu Val Glu Ala
            420                 425                 430

Ala Gly Gly Met Ala Gln Ala Ile Asp Ala Gly Ile Pro Lys Leu Arg
        435                 440                 445
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```
Val Glu Glu Ala Ala Ala Arg Thr Gln Ala Arg Ile Asp Ser Gly Arg
    450                 455                 460

Gln Pro Val Ile Gly Val Asn Lys Tyr Arg Val Asp Thr Asp Glu Gln
465                 470                 475                 480

Ile Asp Val Leu Lys Val Asp Asn Ser Ser Val Arg Ala Gln Gln Ile
                485                 490                 495

Glu Lys Leu Arg Arg Leu Arg Glu Glu Arg Asp Asp Ala Ala Cys Gln
            500                 505                 510

Asp Ala Leu Arg Ala Leu Thr Ala Ala Ala Glu Arg Gly Pro Gly Gln
        515                 520                 525

Gly Leu Glu Gly Asn Leu Leu Ala Leu Ala Val Asp Ala Ala Arg Ala
    530                 535                 540

Lys Ala Thr Val Gly Glu Ile Ser Asp Ala Leu Glu Ser Val Tyr Gly
545                 550                 555                 560

Arg His Ala Gly Gln Ile Arg Thr Ile Ser Gly Val Tyr Arg Thr Glu
                565                 570                 575

Ala Gly Gln Ser Pro Ser Val Glu Arg Thr Arg Ala Leu Val Asp Ala
            580                 585                 590

Phe Asp Glu Ala Glu Gly Arg Arg Pro Arg Ile Leu Val Ala Lys Met
        595                 600                 605

Gly Gln Asp Gly His Asp Arg Gly Gln Lys Val Ile Ala Ser Ala Phe
    610                 615                 620

Ala Asp Leu Gly Phe Asp Val Asp Val Gly Pro Leu Phe Gln Thr Pro
625                 630                 635                 640

Ala Glu Val Ala Arg Gln Ala Val Glu Ala Asp Val His Ile Val Gly
                645                 650                 655

Val Ser Ser Leu Ala Ala Gly His Leu Thr Leu Val Pro Ala Leu Arg
            660                 665                 670

Glu Glu Leu Ala Ala Glu Gly Arg Asp Asp Ile Met Ile Val Val Gly
        675                 680                 685

Gly Val Ile Pro Pro Gln Asp Val Glu Ala Leu His Glu Ala Gly Ala
    690                 695                 700

Thr Ala Val Phe Pro Pro Gly Thr Val Ile Pro Asp Ala Ala His Asp
705                 710                 715                 720

Leu Val Lys Arg Leu Ala Ala Asp Leu Gly His Glu Leu
                725                 730


<210> SEQ ID NO 26
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Streptomyces sviceus

<400> SEQUENCE: 26

Met Leu Thr Arg Ile Asp His Ile Gly Ile Ala Cys Phe Asp Leu Asp
1               5                   10                  15

Lys Thr Val Glu Phe Tyr Arg Ala Thr Tyr Gly Phe Glu Val Phe His
            20                  25                  30

Ser Glu Val Asn Glu Glu Gln Gly Val Arg Glu Ala Met Leu Lys Ile
        35                  40                  45

Asn Glu Thr Ser Asp Gly Gly Ala Ser Tyr Leu Gln Leu Leu Glu Pro
    50                  55                  60

Thr Arg Pro Asp Ser Thr Val Ala Lys Trp Leu Asp Lys Asn Gly Glu
65                  70                  75                  80
```

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```
Gly Val His His Ile Ala Phe Gly Thr Ala Asp Val Asp Gln Asp Ala
                85                  90                  95

Ala Asp Ile Lys Asp Lys Gly Val Arg Val Leu Tyr Glu Glu Pro Arg
            100                 105                 110

Arg Gly Ser Met Gly Ser Arg Ile Thr Phe Leu His Pro Lys Asp Cys
        115                 120                 125

His Gly Val Leu Thr Glu Leu Val Thr Ser Ala Pro Val Glu Ser Pro
    130                 135                 140

Glu His
145
```

<210> SEQ ID NO 27
<211> LENGTH: 4553
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic nucleotide

<400> SEQUENCE: 27

```
gaattcaaaa ttaagaggta tatattaatg accgtgctgc cggatgacgg tctgagtctg     60

gcagccgaat ttccggatgc gacgcatgaa cagtggcacc gtctggttga aggcgtggtt    120

cgcaaatcag gcaaagatgt ctcgggcacc gcagctgaag aagccctgag caccacgctg    180

gaagacggtc tgaccacgcg tccgctgtat acggcacgtg atgcagcacc ggacgctggt    240

tttccgggtt tcgcgccgtt tgtgcgtggc tcagttccgg agggtaacac cccgggcggt    300

tgggatgtgc gtcaacgtta cgcatcggca gacccggcac gtaccaacga agcagtgctg    360

acggatctgg aaaatggtgt taccagcctg tggctgacgc tgggttctgc aggtctgccg    420

gtgaccggtc tggaacgtgc actggatggt gtttatctgg acctggtccc ggtggcactg    480

gatgcaggta gcgaagcagc taccgcagca cgtgaactgc tgcgtctgta cgaagcagct    540

ggtgttgctg atgacgcagt ccgtggcacg ctgggtgcag atccgctggg ccatgaagca    600

cgcaccggtg aaaaaagtac gtcctttgca gcagtggcag aactggcacg tctgtgcggt    660

gaacgttatc cgggtctgcg cgctctgacc gttgatgcgc tgccgtacca tgaagctggc    720

gcgtcagcag ctcaggaact gggcgcttcg ctggcgaccg gtgtggaata tctgcgtgcg    780

ctgcacgata aaggcctggg tgttgaaaaa gccttcgcac agctggaatt tcgcttcgcg    840

gccaccgcgg accaatttct gacgattgcc aaactgcgtg cagctcgtcg cctgtgggca    900

cgtgttgcag aagtcagtgg cgtgccggca gcaggtgcac agcgtcaaca tgcagtcacc    960

tccccggtga tgatgacgcg tcgcgatccg tgggtgaaca tgctgcgtac cacggttgct   1020

tgtctgggtg caggtgtcgg cggtgctgat gcagttaccg tcctgccgtt cgatcacgaa   1080

ctgggtctgc cggacgcctt tgcacgtcgc attgcgcgta ataccagtac gatcctgctg   1140

gaagaatccc atctggcccg tgtcattgat ccggcaggcg gtagctggta tgtggaacgc   1200

ctgaccgatg aactggccca cgcagcttgg gacttttttca aagaaatcga acgtgcagat   1260

ggtcaggtcg cagcactgcg tagcggcctg gtgggtgacc gcattgcagc tacctgggca   1320

gaacgtcgca aaaaactggc gcgtcgccgt gaaccgatca ccggtgtgtc tgaatttccg   1380

ctgctgacgg aacgcccggt tgaacgtgaa ccggcaccgg cagcaccgcc gggcggtctg   1440

ccgcgcgtgc gccgtgatga agcctacgaa gaactgcgtg gtcgttctga cgcacacctg   1500
```

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gaagctaccg gtgcacgtcc gaaagtgttc attgcagctc tgggtccggc agcagcacat 1560

accgctcgtg cgacgttcgc tgcgaacctg tttatggcgg gcggtgttga accggtccac 1620

gatcctgtga gcgttgacgc ggaaaccgcc gcagaagcct ttgctgcgtc tggcgccacg 1680

gttgcatgcc tgtgtagctc tgatgtcctg tatgcggaac aagccgaagc agtcgctcgt 1740

gcgctgaaaa gtgccggtgc actgcgtgtt ttcctggcag gccgcggtga atttgcggat 1800

atcgacgaat acgtgtttgc aggttgcgat gctgtcgcag tgctgacctc cacgctggac 1860

cgtatgggtg ttgcgtaatg cgtattccgg aatttgatga catcgaactg ggtgccggcg 1920

gtggcccgtc aggttcggca gaacagtggc gtgcagcagt gaaagaaagc gttggtaaaa 1980

gcgaatctga tctgctgtgg gaaaccccgg aaggcattgc tgttaaaccg ctgtacacgg 2040

gtgccgatgt cgaaggcctg gacttcctgg aaacctatcc gggtgtcgca ccgtacctgc 2100

gtggtccgta tccgaccatg tacgtgaacc agccgtggac gatccgccaa tacgcgggtt 2160

ttagcaccgc cgaagaatct aacgcattct atcgtcgcaa tctggcagct ggccagaaag 2220

gtctgagtgt ggcgtttgat ctgccgaccc atcgtggcta cgattccgac cacccgcgtg 2280

tcacgggtga cgtgggtatg gccggcgtgg caattgatag catctatgac atgcgtcagc 2340

tgttcgatgg tattccgctg gacaaaatga ccgtttctat gacgatgaac ggcgctgtgc 2400

tgccggttct ggcgctgtat atcgtggcgg ccgaagaaca gggtgttccg ccggaaaaac 2460

tggcgggcac catccaaaac gatatcctga aagaatttat ggttcgtaac acgtacatct 2520

acccgccgaa accgagtatg cgcattatct ccgatatctt cgcctatacc tcacagaaaa 2580

tgccgcgcta caacagtatc tccatctcag gttatcatat ccaagaagca ggcgctaccg 2640

cggatctgga actggcctac acgctggcag acggtgttga atatctgcgt gctggtcagg 2700

aagcgggcct ggatgtcgac gcctttgcac cgcgcctgag ctttttctgg gccattggca 2760

tgaacttttt catggaagtg gcaaaactgc gtgcagctcg cctgctgtgg gcgaaactgg 2820

ttaaacagtt tgatccgaaa aatgcgaaat cgctgagcct gcgtacccac tcccagacgt 2880

caggttggtc gctgaccgcc caagatgttt tcaacaatgt cacccgcacg tgcgtggaag 2940

caatggcagc aacccagggt catacgcaat cactgcacac caacgcgctg gatgaagctc 3000

tggcgctgcc gaccgacttt tcggctcgta ttgcgcgcaa tacgcagctg ctgatccagc 3060

aagaaagcgg caccacgcgt accattgatc cgtggggtgg ctctgcgtat gtggaaaaac 3120

tgacgtacga cctggcacgt cgcgcatggc agcatatcga agaagttgaa gcagcgggtg 3180

gcatggccca agcaattgat gcgggcatcc cgaaactgcg tgtggaagaa gcggcagcac 3240

gtacccaggc acgcattgat tctggtcgtc aaccggtcat cggcgtgaac aaatatcgcg 3300

tggatacgga cgaacagatt gatgttctga aagtcgacaa tagctctgtt cgcgcgcagc 3360

aaatcgaaaa actgcgtcgc ctgcgtgaag aacgcgatga cgctgcgtgt caggatgctc 3420

tgcgtgcact gaccgcagca gctgaacgtg gtccgggtca gggtctggaa ggtaatctgc 3480

tggctctggc agtggatgca gcacgtgcca aagcaaccgt tggcgaaatt tcagacgcac 3540

tggaatcggt ctacggtcgt catgcgggcc agattcgcac catcagtggt gtgtatcgca 3600

cggaagcggg ccaatctccg agtgtcgaac gtacccgcgc cctggtggat gcatttgacg 3660

aagctgaagg tcgtcgcccg cgtattctgg ttgccaaaat gggtcaggat ggccacgacc 3720

gcggccaaaa agtcatcgct tccgcgtttg ccgatctggg tttcgatgtc gacgtgggtc 3780

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cgctgttcca gaccccggcc gaagtggcac gtcaagctgt ggaagcggat gttcatattg 3840

ttggtgtcag ttccctggca gctggtcacc tgacgctggt tccggcactg cgtgaagaac 3900

tggcggccga aggtcgcgat gacattatga tcgtggttgg tggcgtcatt ccgccgcagg 3960

atgtggaagc cctgcatgaa gcaggtgcta ccgcggtttt tccgccgggc acggtcatcc 4020

cggatgcagc tcatgacctg gtgaaacgtc tggcagcaga tctgggtcac gaactgtaaa 4080

agcttaaaat taagaggtat atattaatgc tgacccgcat cgatcacatt ggcatcgcat 4140

gctttgatct ggataaaacc gtagagttct atcgcgccac ctacggcttt gaggtgtttc 4200

atagcgaagt aaacgaagaa cagggcgtgc gtgaagccat gctgaaaatc aacgaaacta 4260

gtgatggtgg ggcgagctat ctgcaactgc tggaaccgac acgcccggac tctacagttg 4320

ctaagtggct ggacaagaat ggcgaaggcg ttcatcacat tgcgttcggt acggctgatg 4380

tggatcaaga cgcggcagat attaaagata agggtgtgcg tgttctgtac gaggagccac 4440

gccgtggtag catgggtagc cgtattacgt tcctgcaccc taaagactgt catggtgtgc 4500

tgactgagct ggtcacctct gccccggtcg aaagtccgga acattaaggt acc 4553

<210> SEQ ID NO 28
<211> LENGTH: 717
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 28

Met Ser Arg Met Ser Asn Val Gln Glu Trp Gln Gln Leu Ala Asn Lys
1 5 10 15

Glu Leu Ser Arg Arg Glu Lys Thr Val Asp Ser Leu Val His Gln Thr
20 25 30

Ala Glu Gly Ile Ala Ile Lys Pro Leu Tyr Thr Glu Ala Asp Leu Asp
35 40 45

Asn Leu Glu Val Thr Gly Thr Leu Pro Gly Leu Pro Pro Tyr Val Arg
50 55 60

Gly Pro Arg Ala Thr Met Tyr Thr Ala Gln Pro Trp Thr Ile Arg Gln
65 70 75 80

Tyr Ala Gly Phe Ser Thr Ala Lys Glu Ser Asn Ala Phe Tyr Arg Arg
85 90 95

Asn Leu Ala Ala Gly Gln Lys Gly Leu Ser Val Ala Phe Asp Leu Ala
100 105 110

Thr His Arg Gly Tyr Asp Ser Asp Asn Pro Arg Val Ala Gly Asp Val
115 120 125

Gly Lys Ala Gly Val Ala Ile Asp Thr Val Glu Asp Met Lys Val Leu
130 135 140

Phe Asp Gln Ile Pro Leu Asp Lys Met Ser Val Ser Met Thr Met Asn
145 150 155 160

Gly Ala Val Leu Pro Val Leu Ala Phe Tyr Ile Val Ala Ala Glu Glu
165 170 175

Gln Gly Val Thr Pro Asp Lys Leu Thr Gly Thr Ile Gln Asn Asp Ile
180 185 190

Leu Lys Glu Tyr Leu Cys Arg Asn Thr Tyr Ile Tyr Pro Pro Lys Pro
195 200 205

Ser Met Arg Ile Ile Ala Asp Ile Ile Ala Trp Cys Ser Gly Asn Met
210 215 220

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Pro Arg Phe Asn Thr Ile Ser Ile Ser Gly Tyr His Met Gly Glu Ala
225                 230                 235                 240

Gly Ala Asn Cys Val Gln Gln Val Ala Phe Thr Leu Ala Asp Gly Ile
                245                 250                 255

Glu Tyr Ile Lys Ala Ala Ile Ser Ala Gly Leu Lys Ile Asp Asp Phe
            260                 265                 270

Ala Pro Arg Leu Ser Phe Phe Phe Gly Ile Gly Met Asp Leu Phe Met
        275                 280                 285

Asn Val Ala Met Leu Arg Ala Ala Arg Tyr Leu Trp Ser Glu Ala Val
    290                 295                 300

Ser Gly Phe Gly Ala Gln Asp Pro Lys Ser Leu Ala Leu Arg Thr His
305                 310                 315                 320

Cys Gln Thr Ser Gly Trp Ser Leu Thr Glu Gln Asp Pro Tyr Asn Asn
                325                 330                 335

Val Ile Arg Thr Thr Ile Glu Ala Leu Ala Ala Thr Leu Gly Gly Thr
            340                 345                 350

Gln Ser Leu His Thr Asn Ala Phe Asp Glu Ala Leu Gly Leu Pro Thr
        355                 360                 365

Asp Phe Ser Ala Arg Ile Ala Arg Asn Thr Gln Ile Ile Ile Gln Glu
    370                 375                 380

Glu Ser Glu Leu Cys Arg Thr Val Asp Pro Leu Ala Gly Ser Tyr Tyr
385                 390                 395                 400

Ile Glu Ser Leu Thr Asp Gln Ile Val Lys Gln Ala Arg Ala Ile Ile
                405                 410                 415

Gln Gln Ile Asp Glu Ala Gly Gly Met Ala Lys Ala Ile Glu Ala Gly
            420                 425                 430

Leu Pro Lys Arg Met Ile Glu Glu Ala Ser Ala Arg Glu Gln Ser Leu
        435                 440                 445

Ile Asp Gln Gly Lys Arg Val Ile Val Gly Val Asn Lys Tyr Lys Leu
    450                 455                 460

Asp His Glu Asp Glu Thr Asp Val Leu Glu Ile Asp Asn Val Met Val
465                 470                 475                 480

Arg Asn Glu Gln Ile Ala Ser Leu Glu Arg Ile Arg Ala Thr Arg Asp
                485                 490                 495

Asp Ala Ala Val Thr Ala Ala Leu Asn Ala Leu Thr His Ala Ala Gln
            500                 505                 510

His Asn Glu Asn Leu Leu Ala Ala Ala Val Asn Ala Ala Arg Val Arg
        515                 520                 525

Ala Thr Leu Gly Glu Ile Ser Asp Ala Leu Glu Val Ala Phe Asp Arg
    530                 535                 540

Tyr Leu Val Pro Ser Gln Cys Val Thr Gly Val Ile Ala Gln Ser Tyr
545                 550                 555                 560

His Gln Ser Glu Lys Ser Ala Ser Glu Phe Asp Ala Ile Val Ala Gln
                565                 570                 575

Thr Glu Gln Phe Leu Ala Asp Asn Gly Arg Arg Pro Arg Ile Leu Ile
            580                 585                 590

Ala Lys Met Gly Gln Asp Gly His Asp Arg Gly Ala Lys Val Ile Ala
        595                 600                 605

Ser Ala Tyr Ser Asp Leu Gly Phe Asp Val Asp Leu Ser Pro Met Phe
    610                 615                 620

Ser Thr Pro Glu Glu Ile Ala Arg Leu Ala Val Glu Asn Asp Val His
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625 630 635 640

Val Val Gly Ala Ser Ser Leu Ala Ala Gly His Lys Thr Leu Ile Pro
645 650 655

Glu Leu Val Glu Ala Leu Lys Lys Trp Gly Arg Glu Asp Ile Cys Val
660 665 670

Val Ala Gly Gly Val Ile Pro Pro Gln Asp Tyr Ala Phe Leu Gln Glu
675 680 685

Arg Gly Val Ala Ala Ile Tyr Gly Pro Gly Thr Pro Met Leu Asp Ser
690 695 700

Val Arg Asp Val Leu Asn Leu Ile Ser Gln His His Asp
705 710 715

<210> SEQ ID NO 29
<211> LENGTH: 2166
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic nucleotide

<400> SEQUENCE: 29

ggatccatgt ctagaatgag caacgtgcag gaatggcagc agctggcgaa taaagaactg 60

agccgtcgcg aaaaaacggt tgattctctg gtgcatcaga ccgccgaagg tatcgcaatt 120

aaaccgctgt ataccgaagc ggatctggat aacctggaag tgaccggtac gctgccgggt 180

ctgccgccgt atgttcgtgg tccgcgtgcg accatgtaca cggcacagcc gtggacgatt 240

cgtcagtatg cgggcttcag caccgccaaa gaatctaacg cattttaccg tcgcaatctg 300

gcggcgggtc agaaaggtct gagcgtggcg tttgatctgg ccacccaccg tggttacgat 360

tctgataacc cgcgcgttgc gggcgatgtg ggtaaagcag gcgttgcgat cgatacggtg 420

gaagatatga aagttctgtt cgatcagatt ccgctggata aaatgagtgt tagcatgacc 480

atgaatggcg cggttctgcc ggtgctggcc ttttatatcg tggcagcgga agaacagggt 540

gttacgccgg ataaactgac cggcacgatc cagaacgata ttctgaaaga atacctgtgc 600

cgtaatacct atatttaccc gccgaaaccg tctatgcgca ttatcgcaga tattatcgcg 660

tggtgtagtg gtaacatgcc gcgtttcaat acgatctcta ttagtggcta tcatatgggt 720

gaagccggcg caaactgcgt tcagcaggtg gcctttaccc tggcagatgg tatcgaatac 780

attaaagccg caatcagtgc gggcctgaaa attgatgatt tcgccccgcg cctgagcttt 840

ttctttggca ttggtatgga tctgtttatg aatgtggcca tgctgcgtgc ggcccgctat 900

ctgtggagcg aagcagtttc tggctttggc gcgcaggacc cgaaaagcct ggcactgcgt 960

acccattgcc agacgagtgg ttggagcctg accgaacagg acccgtacaa caatgtgatc 1020

cgcaccacga ttgaagcgct ggcagcaacc ctgggtggta cgcagagcct gcacaccaac 1080

gcgttcgatg aagccctggg tctgccgacg gattttagcg cccgtatcgc acgcaatacc 1140

cagattatca ttcaggaaga atctgaactg tgtcgtacgg ttgatccgct ggcgggcagt 1200

tattacatcg aaagcctgac cgatcagatt gttaaacagg cgcgtgcgat cattcagcag 1260

attgatgaag caggcggtat ggcaaaagcg atcgaagcgg gcctgccgaa acgtatgatt 1320

gaagaagcct ctgcacgcga acagagtctg atcgatcagg gtaaacgtgt gattgttggc 1380

gtgaacaaat acaaactgga tcatgaagat gaaaccgatg tgctggaaat cgataacgtt 1440

atggtgcgta atgaacagat cgccagcctg gaacgtattc gcgcaacccg cgatgatgcc 1500

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gcagttacgg cggccctgaa cgcactgacc catgcagcgc agcacaacga aaatctgctg 1560

gccgcagcgg tgaatgccgc acgtgttcgc gcgacgctgg gtgaaatttc tgatgcactg 1620

gaagtggcgt tcgatcgcta tctggttccg agtcagtgcg ttaccggcgt gatcgcccag 1680

agttaccatc agagcgaaaa aagcgcatct gaatttgatg cgattgtggc ccagaccgaa 1740

cagtttctgg cagataacgg ccgtcgcccg cgtatcctga ttgccaaaat gggtcaggat 1800

ggccacgatc gcggtgcgaa agtgatcgcg tctgcctata gtgatctggg cttcgatgtt 1860

gatctgtctc cgatgtttag tacgccggaa gaaattgcac gtctggcggt tgaaaatgat 1920

gtgcatgtgg ttggtgccag ctctctggcg gcgggtcaca aaaccctgat tccggaactg 1980

gtggaagcgc tgaaaaaatg gggtcgcgaa gatatctgtg tggttgcggg cggtgtgatt 2040

ccgccgcagg attatgcgtt tctgcaagaa cgtggtgttg cagcaatcta cggtccgggc 2100

accccgatgc tggatagtgt tcgcgatgtg ctgaatctga ttagccagca tcacgattaa 2160

gagctc 2166

<210> SEQ ID NO 30
<211> LENGTH: 1206
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic nucleotide

<400> SEQUENCE: 30

ggatccatga aaataaaaac aggtgcacgc atcctcgcat tatccgcatt aacgacgatg 60

atgttttccg cctcggctct cgccaaaatc gaagaaggta aactggtaat ctggattaac 120

ggcgataaag gctataacgg tctcgctgaa gtcggtaaga aattcgagaa agataccgga 180

attaaagtca ccgttgagca tccggataaa ctggaagaga aattcccaca ggttgcggca 240

actggcgatg gccctgacat tatcttctgg gcacacgacc gctttggtgg ctacgctcaa 300

tctggcctgt tggctgaaat caccccggac aaagcgttcc aggacaagct gtatccgttt 360

acctgggatg ccgtacgtta caacggcaag ctgattgctt acccgatcgc tgttgaagcg 420

ttatcgctga tttataacaa agacctgctg ccgaacccgc caaaaacctg ggaagagatc 480

ccggcgctgg ataaagaact gaaagcgaaa ggtaagagcg cgctgatgtt caacctgcaa 540

gaaccgtact tcacctggcc gctgattgct gctgacgggg gttatgcgtt caagtatgaa 600

aacggcaagt acgacattaa agacgtgggc gtggataacg ctggcgcgaa agcgggtctg 660

accttcctgg ttgacctgat taaaaacaaa cacatgaatg cagacaccga ttactccatc 720

gcagaagctg cctttaataa aggcgaaaca gcgatgacca tcaacggccc gtgggcatgg 780

tccaacatcg acaccagcaa agtgaattat ggtgtaacgg tactgccgac cttcaagggt 840

caaccatcca aaccgttcgt tggcgtgctg agcgcaggta ttaacgccgc cagtccgaac 900

aaagagctgg cgaaagagtt cctcgaaaac tatctgctga ctgatgaagg tctggaagcg 960

gttaataaag acaaaccgct gggtgccgta gcgctgaagt cttacgagga agagttggcg 1020

aaagatccac gtattgccgc caccatggaa aacgcccaga aaggtgaaat catgccgaac 1080

atcccgcaga tgtccgcttt ctggtatgcc gtgcgtactg cggtgatcaa cgccgccagc 1140

ggtcgtcaga ctgtcgatga agccctgaaa gacgcgcaga ctcgtatcac caagtctaga 1200

gagctc 1206

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<210> SEQ ID NO 31
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 31

gagaggtacc atgggggtt ctcatcatca tcatcatc 38

<210> SEQ ID NO 32
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 32

cagccaagct tttattacgc accctgtgcg cgctgttc 38

<210> SEQ ID NO 33
<211> LENGTH: 1263
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic nucleotide

<400> SEQUENCE: 33

atgggggtt ctcatcatca tcatcatcat ggtatggcta gcatgactgg tggacagcaa 60

atgggtcggg atctgtacga cgatgacgat aaggatcgat ggggatccct ggtggaaggc 120

ctgcgtgaag ttgccgatgg tgatgcactg tatgatgcag cagtgggtca tggcgatcgt 180

ggtccggttt gggtgtttag cggccagggt tctcagtggg cagcgatggg cacccagctg 240

ctggcaagcg aaccggtttt tgccgcaacg attgcaaaac tggaaccggt gatcgcggcc 300

gaaagtggct tcagcgttac cgaagcaatt acggcgcagc agaccgtgac gggtatcgat 360

aaagtgcagc cggccgtttt cgcagttcag gtggcgctgg cagcgacgat ggaacagacg 420

tacggcgttc gtccgggtgc agtggttggt cacagtatgg gtgaaagcgc cgcagcggtg 480

gttgcaggcg ccctgagtct ggaagatgcc gcacgtgtga tttgccgtcg cagcaaactg 540

atgacccgta tcgcaggtgc aggtgcgatg ggcagcgtgg aactgccggc aaaacaggtt 600

aactctgaac tgatggcgcg cggtattgat gatgtggttg tgtctgttgt ggcgtctccg 660

cagagtaccg tgattggcgg caccagtgat acggttcgtg atctgatcgc gcgttgggaa 720

cagcgcgatg tgatggcgcg cgaagttgcc gtggatgttg caagccattc tccgcaggtt 780

gatccgattc tggatgatct ggcggcggca ctggcagata ttgcaccgat gaccccgaaa 840

gtgccgtatt acagcgcgac gctgtttgat ccgcgtgaac agccggtgtg tgatggcgcc 900

tattgggttg ataacctgcg caataccgtg cagtttgcgg cggcagttca ggcggcgatg 960

gaagatggtt accgtgtgtt cgcggaactg tctccgcatc cgctgctgac ccacgcagtg 1020

gaacagacgg gtcgctctct ggatatgagt gttgcagcac tggccggtat gcgtcgcgaa 1080

cagccgctgc cgcatggcct gcgtggtctg ctgaccgaac tgcaccgtgc aggtgcagca 1140

ctggattata gcgcactgta cccggcaggt cgtctggtgg atgcaccgct gccggcatgg 1200

acgcacgcac gtctgttcat cgatgatgat ggccaggaac agcgcgcaca gggtgcgtaa 1260

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taa 1263

<210> SEQ ID NO 34
<211> LENGTH: 419
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium bovis

<400> SEQUENCE: 34

Met Gly Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
20 25 30

Arg Trp Gly Ser Leu Val Glu Gly Leu Arg Glu Val Ala Asp Gly Asp
35 40 45

Ala Leu Tyr Asp Ala Ala Val Gly His Gly Asp Arg Gly Pro Val Trp
50 55 60

Val Phe Ser Gly Gln Gly Ser Gln Trp Ala Ala Met Gly Thr Gln Leu
65 70 75 80

Leu Ala Ser Glu Pro Val Phe Ala Ala Thr Ile Ala Lys Leu Glu Pro
85 90 95

Val Ile Ala Ala Glu Ser Gly Phe Ser Val Thr Glu Ala Ile Thr Ala
100 105 110

Gln Gln Thr Val Thr Gly Ile Asp Lys Val Gln Pro Ala Val Phe Ala
115 120 125

Val Gln Val Ala Leu Ala Ala Thr Met Glu Gln Thr Tyr Gly Val Arg
130 135 140

Pro Gly Ala Val Val Gly His Ser Met Gly Glu Ser Ala Ala Ala Val
145 150 155 160

Val Ala Gly Ala Leu Ser Leu Glu Asp Ala Ala Arg Val Ile Cys Arg
165 170 175

Arg Ser Lys Leu Met Thr Arg Ile Ala Gly Ala Gly Ala Met Gly Ser
180 185 190

Val Glu Leu Pro Ala Lys Gln Val Asn Ser Glu Leu Met Ala Arg Gly
195 200 205

Ile Asp Asp Val Val Val Ser Val Val Ala Ser Pro Gln Ser Thr Val
210 215 220

Ile Gly Gly Thr Ser Asp Thr Val Arg Asp Leu Ile Ala Arg Trp Glu
225 230 235 240

Gln Arg Asp Val Met Ala Arg Glu Val Ala Val Asp Val Ala Ser His
245 250 255

Ser Pro Gln Val Asp Pro Ile Leu Asp Asp Leu Ala Ala Ala Leu Ala
260 265 270

Asp Ile Ala Pro Met Thr Pro Lys Val Pro Tyr Tyr Ser Ala Thr Leu
275 280 285

Phe Asp Pro Arg Glu Gln Pro Val Cys Asp Gly Ala Tyr Trp Val Asp
290 295 300

Asn Leu Arg Asn Thr Val Gln Phe Ala Ala Ala Val Gln Ala Ala Met
305 310 315 320

Glu Asp Gly Tyr Arg Val Phe Ala Glu Leu Ser Pro His Pro Leu Leu
325 330 335

Thr His Ala Val Glu Gln Thr Gly Arg Ser Leu Asp Met Ser Val Ala
340 345 350

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```
Ala Leu Ala Gly Met Arg Arg Glu Gln Pro Leu Pro His Gly Leu Arg
        355                 360                 365

Gly Leu Leu Thr Glu Leu His Arg Ala Gly Ala Ala Leu Asp Tyr Ser
    370                 375                 380

Ala Leu Tyr Pro Ala Gly Arg Leu Val Asp Ala Pro Leu Pro Ala Trp
385                 390                 395                 400

Thr His Ala Arg Leu Phe Ile Asp Asp Asp Gly Gln Glu Gln Arg Ala
                405                 410                 415

Gln Gly Ala
```

<210> SEQ ID NO 35
<211> LENGTH: 464
<212> TYPE: DNA
<213> ORGANISM: Kribbella flavida DSM

<400> SEQUENCE: 35

```
gagctcagga ggaattaacc atggaacacc tgacggcgac ccagaccctg tttgaagcga     60

ttgaccacgt tggcgttgca gttgcggatt ttgatgaagc agtgcgtttt tatgcagaaa    120

ccttcggcat gacggtggct catgaagaag ttaacgaaga acagggtgtt cgtgaagcaa    180

tgctgtcaat tggcgattcg ggtagctcta tccaactgct ggcgccgctg tccgatagtt    240

ccccgattgc caaatttctg gaccgcaatg gcccgggtat ccagcaactg gcctatcgtg    300

tccgcgatct ggacgcagtg agcgcaaccc tgcgtgaacg tggcgcgcaa ctgctgtacg    360

acgaaccgcg tcgcggcacg gctggttctc gtattaactt cattcatccg aaatcggcgg    420

gcggcgtcct ggtggaactg gtggaaccgg ctcgctaact gcag                     464
```

<210> SEQ ID NO 36
<211> LENGTH: 145
<212> TYPE: PRT
<213> ORGANISM: Kribbella flavida DSM

<400> SEQUENCE: 36

```
Met Glu His Leu Thr Ala Thr Gln Thr Leu Phe Glu Ala Ile Asp His
1               5                   10                  15

Val Gly Val Ala Val Ala Asp Phe Asp Glu Ala Val Arg Phe Tyr Ala
            20                  25                  30

Glu Thr Phe Gly Met Thr Val Ala His Glu Glu Val Asn Glu Glu Gln
        35                  40                  45

Gly Val Arg Glu Ala Met Leu Ser Ile Gly Asp Ser Gly Ser Ser Ile
    50                  55                  60

Gln Leu Leu Ala Pro Leu Ser Asp Ser Ser Pro Ile Ala Lys Phe Leu
65                  70                  75                  80

Asp Arg Asn Gly Pro Gly Ile Gln Gln Leu Ala Tyr Arg Val Arg Asp
                85                  90                  95

Leu Asp Ala Val Ser Ala Thr Leu Arg Glu Arg Gly Ala Gln Leu Leu
            100                 105                 110

Tyr Asp Glu Pro Arg Arg Gly Thr Ala Gly Ser Arg Ile Asn Phe Ile
        115                 120                 125

His Pro Lys Ser Ala Gly Gly Val Leu Val Glu Leu Val Glu Pro Ala
    130                 135                 140

Arg
145
```

-continued

<210> SEQ ID NO 37
<211> LENGTH: 545
<212> TYPE: DNA
<213> ORGANISM: Sorangium cellulosum

<400> SEQUENCE: 37

gagctcagga ggaattaacc atggctccgc cggcaacgcg tccggctccg gctgcaccga 60

cgggcctgcc gacccaacgt gaaccgatga aagaccagat tccgggcttt ctgttcattg 120

atcatatcgc gatggccgtg ccggcaggcc aactggacgc acaagttaaa gcctatgaaa 180

tgctgggctt tcgtgaagtt catcgcgaag aagtccgtgg tgcggatcag gtgcgcgaag 240

ttatgctgcg tattggtgat agcgacaacc acgtccaact gctggaaccg ctgagcccgg 300

aatctccggt tcaaaaactg atcgagaaaa acggcggtcg cggcggtttc gcacatgtgg 360

cttaccgtgt cagtgatgtg caagcggcct ttgacgaact gaaagcgcgt ggcttccgca 420

ttatcgatgc agctccgcgt ccgggcagcc gtggcaccac gattttcttt gttcacccgc 480

gctcacgcga cgatgccccg ttcggtcacc tgattgaagt tgtccagtca catggctaac 540

tgcag 545

<210> SEQ ID NO 38
<211> LENGTH: 172
<212> TYPE: PRT
<213> ORGANISM: Sorangium cellulosum

<400> SEQUENCE: 38

Met Ala Pro Pro Ala Thr Arg Pro Ala Pro Ala Ala Pro Thr Gly Leu
1 5 10 15

Pro Thr Gln Arg Glu Pro Met Lys Asp Gln Ile Pro Gly Phe Leu Phe
20 25 30

Ile Asp His Ile Ala Met Ala Val Pro Ala Gly Gln Leu Asp Ala Gln
35 40 45

Val Lys Ala Tyr Glu Met Leu Gly Phe Arg Glu Val His Arg Glu Glu
50 55 60

Val Arg Gly Ala Asp Gln Val Arg Glu Val Met Leu Arg Ile Gly Asp
65 70 75 80

Ser Asp Asn His Val Gln Leu Leu Glu Pro Leu Ser Pro Glu Ser Pro
85 90 95

Val Gln Lys Leu Ile Glu Lys Asn Gly Gly Arg Gly Gly Phe Ala His
100 105 110

Val Ala Tyr Arg Val Ser Asp Val Gln Ala Ala Phe Asp Glu Leu Lys
115 120 125

Ala Arg Gly Phe Arg Ile Ile Asp Ala Ala Pro Arg Pro Gly Ser Arg
130 135 140

Gly Thr Thr Ile Phe Phe Val His Pro Arg Ser Arg Asp Asp Ala Pro
145 150 155 160

Phe Gly His Leu Ile Glu Val Val Gln Ser His Gly
165 170

<210> SEQ ID NO 39
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 39

taagagctca ggaggaatta accatg 26

<210> SEQ ID NO 40
<211> LENGTH: 566
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 40

catgccatgg cggacacgtt attgattctg ggtgatagcc tgagcgccgg gtatcgaatg 60

tctgccagcg cggcctggcc tgccttgttg aatgataagt ggcagagtaa aacgtcggta 120

gttaatgcca gcatcagcgg cgacacctcg caacaaggac tggcgcgcct tccggctctg 180

ctgaaacagc atcagccgcg ttgggtgctg gttgaactgg gcggcaatga cggtttgcgt 240

ggttttcagc cacagcaaac cgagcaaacg ctgcgccaga ttttgcagga tgtcaaagcc 300

gccaacgctg aaccattgtt aatgcaaata cgtctgcctg caaactatgg tcgccgttat 360

aatgaagcct ttagcgccat ttaccccaaa ctcgccaaag agtttgatgt tccgctgctg 420

ccctttttta tggaagaggt ctacctcaag ccacaatgga tgcaggatga cggtattcat 480

cccaaccgcg acgcccagcc gtttattgcc gactggatgg cgaagcagtt gcagccttta 540

gtaaatcatg actcataagg atccgc 566

<210> SEQ ID NO 41
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 41

catgccatgg cggacacgtt attgattctg gg 32

<210> SEQ ID NO 42
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 42

Met Ala Asp Thr Leu Leu Ile Leu Gly
1 5

<210> SEQ ID NO 43
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 43

gcggatcctt atgagtcatg atttactaaa ggctgc 36

<210> SEQ ID NO 44
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 44

Ser Asp His Asn Val Leu Pro Gln Leu
1 5

<210> SEQ ID NO 45
<211> LENGTH: 183
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 45

Met Ala Asp Thr Leu Leu Ile Leu Gly Asp Ser Leu Ser Ala Gly Tyr
1 5 10 15

Arg Met Ser Ala Ser Ala Ala Trp Pro Ala Leu Leu Asn Asp Lys Trp
20 25 30

Gln Ser Lys Thr Ser Val Val Asn Ala Ser Ile Ser Gly Asp Thr Ser
35 40 45

Gln Gln Gly Leu Ala Arg Leu Pro Ala Leu Leu Lys Gln His Gln Pro
50 55 60

Arg Trp Val Leu Val Glu Leu Gly Gly Asn Asp Gly Leu Arg Gly Phe
65 70 75 80

Gln Pro Gln Gln Thr Glu Gln Thr Leu Arg Gln Ile Leu Gln Asp Val
85 90 95

Lys Ala Ala Asn Ala Glu Pro Leu Leu Met Gln Ile Arg Leu Pro Ala
100 105 110

Asn Tyr Gly Arg Arg Tyr Asn Glu Ala Phe Ser Ala Ile Tyr Pro Lys
115 120 125

Leu Ala Lys Glu Phe Asp Val Pro Leu Leu Pro Phe Phe Met Glu Glu
130 135 140

Val Tyr Leu Lys Pro Gln Trp Met Gln Asp Asp Gly Ile His Pro Asn
145 150 155 160

Arg Asp Ala Gln Pro Phe Ile Ala Asp Trp Met Ala Lys Gln Leu Gln
165 170 175

Pro Leu Val Asn His Asp Ser
180

<210> SEQ ID NO 46
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 46

cattactcga gcgcactccc gttctggata atg 33

<210> SEQ ID NO 47
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 47

gggaagctta tgagtcatga tttactaaag gctgc 35

-continued

<210> SEQ ID NO 48
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 48

Ser Asp His Asn Val Leu Pro Gln Leu
1 5

<210> SEQ ID NO 49
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic nucleotide

<400> SEQUENCE: 49

ggatccatgt ctaga 15

<210> SEQ ID NO 50
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 50

Met Gly Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Thr Asp Asp Asp Asp Lys Asp Arg Trp
20 25 30

Gly Ser

<210> SEQ ID NO 51
<211> LENGTH: 655
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia chaffeensis
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_507303
<309> DATABASE ENTRY DATE: 2010-05-14
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(655)

<400> SEQUENCE: 51

Met Ile Lys Lys Ile Leu Ile Ala Asn Arg Gly Glu Ile Ala Cys Arg
1 5 10 15

Val Met Arg Thr Ala Arg Lys Met Gly Ile Ser Cys Val Ala Val Tyr
20 25 30

Ser Asn Ala Asp Val Tyr Ser Leu His Val Leu Ser Ala Glu Glu Ala
35 40 45

Val Asn Ile Gly Pro Ala Pro Val Asn Gln Ser Tyr Leu Asn Met Glu
50 55 60

Lys Ile Cys Glu Val Ala Cys Asn Thr Gly Val Asp Ala Val His Pro
65 70 75 80

Gly Tyr Gly Phe Leu Ser Glu Asn Ala Asp Phe Pro Glu Lys Leu Glu
85 90 95

Gln Tyr Asn Ile Lys Phe Ile Gly Pro Ser Ser Thr Ser Ile Arg Met
100 105 110

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Met Ala Asp Lys Ile Thr Ser Lys Lys Ile Ala Glu Ser Ala Lys Val
        115                 120                 125

Asn Ile Ile Pro Gly Tyr Met Gly Ile Val Asp Ser Val His Glu Ala
    130                 135                 140

Lys Glu Ile Ala Lys Ser Ile Gly Phe Pro Val Met Ile Lys Ala Thr
145                 150                 155                 160

Ala Gly Gly Gly Gly Lys Gly Met Arg Ile Val Lys Ser Ser Glu Glu
                165                 170                 175

Ile Glu Gln Ala Phe Thr Ser Ala Thr Asn Glu Ala Ala Lys Asn Phe
            180                 185                 190

Arg Asp Gly Arg Ile Phe Ile Glu Lys Tyr Val Glu Leu Pro Arg His
        195                 200                 205

Ile Glu Ile Gln Ile Ile Ala Asp Lys His Gly Asn Ile Val Cys Leu
    210                 215                 220

Gly Glu Arg Glu Cys Ser Ile Gln Arg His Asn Gln Lys Val Ile Glu
225                 230                 235                 240

Glu Thr Pro Ser Pro Phe Leu Asp Glu Glu Thr Arg Gln Lys Met Tyr
                245                 250                 255

Gln Gln Cys Val Asn Leu Ala Lys Lys Val Gly Tyr Tyr Ser Ala Gly
            260                 265                 270

Thr Ile Glu Phe Ile Val Asp Gln Asp Lys Gln Phe Tyr Phe Leu Glu
        275                 280                 285

Met Asn Thr Arg Leu Gln Val Glu His Pro Val Thr Glu Leu Val Thr
    290                 295                 300

Gly Ile Asp Ile Val Glu Glu Met Ile Arg Ile Ala Asp Gly Glu Glu
305                 310                 315                 320

Leu Arg Phe Thr Gln Gln Asp Val Lys Phe Thr Gly Ser Ala Ile Glu
                325                 330                 335

Ala Arg Val Tyr Ala Glu Asn Pro Thr Lys Asn Phe Leu Pro Ser Ser
            340                 345                 350

Gly Arg Ile Ala Tyr Tyr Ser Ala Pro Met Pro Asn Asp Asn Leu Arg
        355                 360                 365

Ile Asp Ser Gly Val Phe Glu Gly Ala Glu Val Ser Met Phe Tyr Asp
    370                 375                 380

Pro Met Ile Ala Lys Val Cys Thr Tyr Gly Lys Asn Arg Asp Glu Ala
385                 390                 395                 400

Val Ser Phe Met Gln Arg Tyr Leu Asn Glu Phe Tyr Ile Gly Gly Ile
                405                 410                 415

Ala Asn Asn Ile Asp Phe Leu Leu Ser Val Phe His His Pro Val Phe
            420                 425                 430

Ile Ser Gly Asn Ile Asn Thr Lys Phe Ile Glu Gln Phe Tyr Phe Asp
        435                 440                 445

Gly Phe Gln Gly Asn Pro Leu Thr Lys Ala Cys Ile Lys Leu Phe Ile
    450                 455                 460

Leu Thr Ser Leu Cys Ile Phe Phe Gln Asp Glu Tyr Gly Ile His Gly
465                 470                 475                 480

Val Glu Leu Cys Glu Asn Arg Glu Leu Ala Val Tyr Val Asp Gly Gln
                485                 490                 495

Lys Tyr Leu Ile Ser Ala Lys Tyr Glu Asn Gly Arg Val Leu Ala Ile
            500                 505                 510

Tyr Asp Gln Cys Glu Tyr Leu Val Val Ser Thr Trp Asn Val Asn Phe
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        515                 520                 525

Lys Ile Leu Gln Ile Gln Val Asn Asn Asp Glu Val Phe His Val Lys
    530                 535                 540

Val Asp Ser Arg Leu Asn Lys Tyr Gln Leu Lys Tyr Ser Ala Met Ser
545                 550                 555                 560

Ala Leu Cys Ala Val Tyr Lys Pro Cys Val Ser Asp Leu Leu Pro Ile
                565                 570                 575

Met Pro Gln Ile Ser Gly Glu Glu Leu Tyr Ser Ser Asn Val Cys Ser
            580                 585                 590

Pro Ile Ser Gly Met Ile Val Lys Ile Tyr Val Lys Gln Gly Glu Glu
        595                 600                 605

Val Gln Pro Gly Gln Pro Leu Leu Val Ile Glu Ala Met Lys Met Glu
    610                 615                 620

Asn Val Ile Tyr Ser Asp Val Lys Ser Ile Val Lys Ser Val Leu Phe
625                 630                 635                 640

Ser Glu Gly Asn Ser Val Ala Thr Gly Asp Val Ile Ile Glu Phe
                645                 650                 655


<210> SEQ ID NO 52
<211> LENGTH: 510
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia chaffeensis
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_507410
<309> DATABASE ENTRY DATE: 2010-05-14
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(510)

<400> SEQUENCE: 52

Met Asn Phe Ala Gly Leu Gln Asp Leu Asn Asn Arg Gln Ser Lys Ser
1                 5                  10                  15

Tyr Asn Gly Gly Gly Leu Ser Arg Ile Glu Lys Gln His Leu Lys Gly
            20                  25                  30

Lys Leu Thr Ala Arg Glu Arg Leu Thr Val Leu Leu Asp Asp Asn Ser
        35                  40                  45

Phe Glu Glu Tyr Gly Ala Phe Val Glu His Arg Cys Val Asn Phe Ser
    50                  55                  60

Met Asp Lys Ser Lys Ile Pro Gly Asp Gly Val Val Val Gly Tyr Gly
65                  70                  75                  80

Thr Ile Asn Gly Arg Lys Val Cys Ile Tyr Ser Gln Asp Phe Thr Val
                85                  90                  95

Phe Gly Gly Ser Leu Ser Glu Ser Asn Ala Lys Lys Ile Cys Asn Ile
            100                 105                 110

Met Asp Lys Ala Ala Ser Leu Gly Ile Pro Ile Ile Gly Ile Asn Asp
        115                 120                 125

Ser Gly Gly Ala Arg Ile Gln Glu Gly Val Asp Ser Leu Ser Gly Tyr
    130                 135                 140

Gly Glu Ile Phe Gln Arg Asn Val Asn Leu Ser Gly Val Val Pro Gln
145                 150                 155                 160

Ile Ser Leu Ile Met Gly Pro Cys Ala Gly Gly Ala Val Tyr Ser Pro
                165                 170                 175

Ala Leu Thr Asp Phe Ile Phe Met Val Arg Asn Thr Ser Tyr Met Phe
            180                 185                 190

Val Thr Gly Pro Asp Val Ile Lys Lys Val Thr Tyr Glu Glu Val Thr
        195                 200                 205
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Gln Glu Asp Leu Gly Gly Ala Lys Val His Ala Ser Lys Thr Gly Ile
    210                 215                 220

Ala Asp Leu Val Phe His Asn Glu Ile Glu Ala Leu Leu Gln Val Arg
225                 230                 235                 240

Arg Phe Met Asn Phe Ile Pro Ser Asn Asn Met Glu Ser Ile Gly Ser
                245                 250                 255

Gln Ser Ala Ser Asn Phe Ile Asn Met Glu Asp Leu Ser Leu Asn Thr
            260                 265                 270

Leu Val Pro Lys Asn Ser Thr Thr Pro Tyr Asn Met Tyr Glu Leu Leu
        275                 280                 285

Glu Lys Val Cys Asp Glu Arg Leu Phe Tyr Glu Ile Lys Pro Asp Phe
    290                 295                 300

Ala Arg Asn Ile Ile Ile Gly Phe Gly Lys Ile Gly Gly Tyr Asn Val
305                 310                 315                 320

Gly Leu Val Ala Asn Gln Pro Leu His Leu Ala Gly Cys Leu Asp Ile
                325                 330                 335

Asp Ala Ser Arg Lys Gly Ala Arg Phe Ile Arg Phe Cys Asp Ala Phe
            340                 345                 350

Asn Ile Pro Val Ile Thr Phe Ile Asp Val Pro Gly Phe Met Pro Gly
        355                 360                 365

Val Asn Gln Glu His Ser Gly Ile Ile Ala His Gly Ala Lys Leu Leu
    370                 375                 380

Tyr Ala Tyr Ala Glu Ala Thr Val Pro Lys Ile Ser Val Ile Val Arg
385                 390                 395                 400

Lys Ala Tyr Gly Gly Ala Tyr Ile Val Met Asn Ser Lys His Leu Cys
                405                 410                 415

Gly Asp Val Asn Tyr Ala Trp Gln Asp Ala Glu Ile Ala Val Met Gly
            420                 425                 430

Ala Glu Gly Ala Val Glu Ile Ile Phe Arg Asn Glu Lys Asp Lys Asp
        435                 440                 445

Lys Ile Gln His Ile Ile Asp Glu Tyr Arg Thr Thr Ile Val Asn Pro
    450                 455                 460

Tyr Val Ala Ala Ser Arg Gly Tyr Ile Asp Asp Ile Ile Val Pro Ser
465                 470                 475                 480

Arg Thr Arg Glu His Leu Phe Lys Ser Leu Gln Phe Leu Glu Lys Lys
                485                 490                 495

Lys Val His Lys Ile Met Arg Lys His Asp Asn Leu Pro Leu
            500                 505                 510


<210> SEQ ID NO 53
<211> LENGTH: 666
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium vitis
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_002547482
<309> DATABASE ENTRY DATE: 2010-04-01
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(666)

<400> SEQUENCE: 53

Met Ala Ile Ser Lys Ile Leu Ile Ala Asn Arg Gly Glu Ile Ala Cys
1               5                   10                  15

Arg Val Ile Lys Thr Ala Lys Arg Met Gly Ile Ala Thr Val Ala Val
            20                  25                  30
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Tyr Ser Asp Ala Asp Ala Asn Ala Leu His Val Lys Leu Ala Asp Glu
        35                  40                  45

Ala Val His Ile Gly Pro Ser Pro Ser Asn Gln Ser Tyr Ile Val Ile
    50                  55                  60

Asp Lys Ile Leu Glu Ala Ile Arg Gln Thr Gly Ala Asp Ala Val His
65                  70                  75                  80

Pro Gly Tyr Gly Phe Leu Ser Glu Asn Ala Ala Phe Ala Glu Ala Leu
                85                  90                  95

Asp Lys Ala Gly Val Ala Phe Ile Gly Pro Pro Val Gly Ala Ile Lys
            100                 105                 110

Ala Met Gly Asp Lys Ile Thr Ser Lys Lys Leu Ala Ala Glu Ala Gly
        115                 120                 125

Val Ser Thr Val Pro Gly His Met Gly Leu Ile Ala Asp Ala Asp Glu
    130                 135                 140

Ala Val Lys Ile Ala Ala Gln Ile Gly Tyr Pro Val Met Ile Lys Ala
145                 150                 155                 160

Ser Ala Gly Gly Gly Gly Lys Gly Met Arg Ile Ala Trp Asn Asp Ala
                165                 170                 175

Glu Ala Arg Glu Gly Phe Gln Ser Ser Lys Asn Glu Ala Met Asn Ser
            180                 185                 190

Phe Gly Asp Asp Arg Ile Phe Ile Glu Lys Phe Val Asp Gln Pro Arg
        195                 200                 205

His Ile Glu Ile Gln Val Leu Gly Asp Lys His Gly Asn Val Leu Tyr
    210                 215                 220

Leu Gly Glu Arg Glu Cys Ser Ile Gln Arg Arg Asn Gln Lys Val Ile
225                 230                 235                 240

Glu Glu Ala Pro Ser Pro Phe Leu Asp Ala Asp Thr Arg Lys Ala Met
                245                 250                 255

Gly Glu Gln Ala Val Ala Leu Ala Lys Ala Val Gly Tyr Tyr Ser Ala
            260                 265                 270

Gly Thr Val Glu Phe Ile Val Asp Gly Asn Arg Asn Phe Tyr Phe Leu
        275                 280                 285

Glu Met Asn Thr Arg Leu Gln Val Glu His Pro Val Thr Glu Leu Ile
    290                 295                 300

Thr Gly Leu Asp Leu Val Glu Gln Met Ile Arg Val Ala Ser Gly Glu
305                 310                 315                 320

Thr Leu Ala Leu Ala Gln Gly Asp Val Thr Leu Thr Gly Trp Ala Val
                325                 330                 335

Glu Ser Arg Leu Tyr Ala Glu Asp Pro Tyr Arg Asn Phe Leu Pro Ser
            340                 345                 350

Ile Gly Arg Leu Ser Arg Tyr Arg Pro Pro Ser Glu Gly Gln Gln Ala
        355                 360                 365

Asp Gly Thr Val Val Arg Asn Asp Thr Gly Val Phe Glu Gly Gly Glu
    370                 375                 380

Ile Ser Met Tyr Tyr Asp Pro Met Val Ala Lys Leu Cys Thr Trp Gly
385                 390                 395                 400

Pro Asp Arg Ile Thr Ala Ile Asp Ala Met Ser Ala Ala Leu Asp Arg
                405                 410                 415

Phe Glu Val Glu Gly Ile Gly His Asn Leu Pro Phe Leu Ser Ala Val
            420                 425                 430

Met Gln His Pro Arg Phe Arg Ser Gly Lys Ile Thr Thr Ala Phe Ile
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        435                 440                 445

Ala Glu Glu Phe Pro Glu Gly Phe Ser Gly Val Glu Pro Asp Glu Met
    450                 455                 460

Ala Gly Lys Thr Leu Ala Ala Ile Ala Ala Leu Val His Gln Arg Arg
465                 470                 475                 480

Glu Ala Arg Ala Ala Gln Val Ser Gly Thr Met Gly Asn His Ala Arg
                485                 490                 495

Thr Ile Gly Arg Asp Trp Val Val Gly Leu Ala Glu Gln Asn Tyr Pro
            500                 505                 510

Leu Thr Leu Ser Thr Asp Pro Gly Ser Met Met Phe Ala Asp Gly Asn
        515                 520                 525

Val Leu Ser Val Asp Gly Val Trp Gln Pro Gly Gln Thr Leu Ala Ile
    530                 535                 540

Phe Thr Val Asn Gly Gln Ser Ile Gly Leu Lys Ile Asp Leu Lys Gly
545                 550                 555                 560

Pro Ala Ile Arg Leu Arg Trp Arg Gly Met Asp Val Val Ala His Val
                565                 570                 575

Arg Asn Pro Arg Val Ala Glu Leu Ala Arg Leu Met Pro Arg Lys Leu
            580                 585                 590

Pro Pro Asp Thr Ser Lys Met Leu Leu Cys Pro Met Pro Gly Val Val
        595                 600                 605

Thr Gly Ile Ala Val Ala Glu Gly Asp Ala Val Glu Ala Gly Gln Ala
    610                 615                 620

Leu Ala Thr Val Glu Ala Met Lys Met Glu Asn Ile Leu Lys Ala Glu
625                 630                 635                 640

Arg Arg Gly Val Val Lys Arg Leu Val Ala Lys Ala Gly Gln Ser Leu
                645                 650                 655

Ala Val Asp Glu Leu Ile Met Glu Phe Glu
            660                 665


<210> SEQ ID NO 54
<211> LENGTH: 510
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium vitis
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_002547479
<309> DATABASE ENTRY DATE: 2010-04-01
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(510)

<400> SEQUENCE: 54

Met Pro Thr Ile Leu Asp Gln Leu Glu Ser Arg Arg Ala Glu Ala Arg
1               5                   10                  15

Leu Gly Gly Gly Glu Lys Arg Ile Asp Ala Gln His Ala Lys Gly Lys
            20                  25                  30

Leu Thr Ala Arg Glu Arg Ile Glu Ile Leu Leu Asp Glu Gly Ser Phe
        35                  40                  45

Glu Glu Tyr Asp Met Tyr Val Thr His Arg Cys Ala Asp Phe Gly Met
    50                  55                  60

Asp Gly Gln Lys Val Ala Gly Asp Gly Val Val Thr Gly Trp Gly Thr
65                  70                  75                  80

Ile Asn Gly Arg Gln Val Tyr Val Phe Ser Gln Asp Phe Thr Val Leu
                85                  90                  95

Gly Gly Ser Leu Ser Glu Thr His Ala Gln Lys Ile Cys Lys Ile Met
            100                 105                 110
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Asp Met Ala Val Arg Val Gly Ala Pro Val Ile Gly Ile Asn Asp Ser
        115                 120                 125

Gly Gly Ala Arg Ile Gln Glu Gly Val Ala Ser Leu Ala Gly Tyr Ala
    130                 135                 140

Glu Val Phe Arg Arg Asn Ala Glu Val Ser Gly Val Ile Pro Gln Ile
145                 150                 155                 160

Ser Val Ile Met Gly Pro Cys Ala Gly Gly Ala Val Tyr Ser Pro Ala
                165                 170                 175

Met Thr Asp Phe Ile Phe Met Val Arg Asp Thr Ser Tyr Met Phe Val
            180                 185                 190

Thr Gly Pro Asp Val Val Lys Thr Val Thr Asn Glu Ile Val Thr Ala
        195                 200                 205

Glu Glu Leu Gly Gly Ala Gly Thr His Thr Lys Lys Ser Ser Val Ala
    210                 215                 220

Asp Gly Ala Phe Glu Asn Asp Val Glu Ala Leu Glu Gln Val Arg Leu
225                 230                 235                 240

Leu Phe Asp Phe Leu Pro Leu Asn Asn Arg Glu Lys Pro Pro Lys Arg
                245                 250                 255

Pro Phe Tyr Asp Asp Pro Ala Arg Leu Glu Met Arg Leu Asp Thr Leu
            260                 265                 270

Ile Pro Asp Ser Ser Thr Lys Pro Tyr Asp Met Lys Glu Leu Ile His
        275                 280                 285

Ala Leu Ala Asp Glu Gly Asp Phe Phe Glu Leu Gln Glu Ala Phe Ala
    290                 295                 300

Lys Asn Ile Ile Thr Gly Phe Ile Arg Leu Glu Gly Gln Thr Val Gly
305                 310                 315                 320

Val Val Ala Asn Gln Pro Met Val Leu Ala Gly Cys Leu Asp Ile Asp
                325                 330                 335

Ser Ser Arg Lys Ala Ala Arg Phe Val Arg Phe Cys Asp Ala Phe Ser
            340                 345                 350

Ile Pro Ile Leu Thr Leu Val Asp Val Pro Gly Phe Leu Pro Gly Val
        355                 360                 365

Ala Gln Glu Tyr Gly Gly Val Ile Lys His Gly Ala Lys Leu Leu Phe
    370                 375                 380

Ala Tyr Ser Glu Ala Thr Val Pro Met Val Thr Leu Ile Thr Arg Lys
385                 390                 395                 400

Ala Tyr Gly Gly Ala Tyr Asp Val Met Ala Ser Lys His Ile Gly Ala
                405                 410                 415

Asp Val Asn Tyr Ala Trp Pro Thr Ala Glu Ile Ala Val Met Gly Ala
            420                 425                 430

Lys Gly Ala Thr Glu Ile Leu Tyr Arg Ser Glu Leu Ala Asp Pro Glu
        435                 440                 445

Lys Ile Ala Ala Arg Thr Arg Glu Tyr Glu Glu Arg Phe Ala Asn Pro
    450                 455                 460

Phe Val Ala Ala Glu Arg Gly Phe Ile Asp Glu Val Ile Met Pro His
465                 470                 475                 480

Ser Ser Arg Lys Arg Ile Ala Arg Ala Phe Ala Ser Leu Arg Gly Lys
                485                 490                 495

Gln Val Ala Thr His Trp Lys Lys His Asp Thr Ile Pro Leu
            500                 505                 510
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<210> SEQ ID NO 55
<211> LENGTH: 667
<212> TYPE: PRT
<213> ORGANISM: Methylobacterium extorquens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_003069256
<309> DATABASE ENTRY DATE: 2010-04-16
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(667)

<400> SEQUENCE: 55

Met Phe Asp Lys Ile Leu Ile Ala Asn Arg Gly Glu Ile Ala Cys Arg
1 5 10 15

Ile Ile Lys Thr Ala Gln Lys Met Gly Ile Lys Thr Val Ala Val Tyr
            20 25 30

Ser Asp Ala Asp Arg Asp Ala Val His Val Ala Met Ala Asp Glu Ala
        35 40 45

Val Asn Ile Gly Pro Ala Pro Ala Ala Gln Ser Tyr Leu Leu Ile Glu
    50 55 60

Lys Ile Ile Asp Ala Cys Lys Gln Thr Gly Ala Gln Ala Val His Pro
65 70 75 80

Gly Tyr Gly Phe Leu Ser Glu Arg Glu Ser Phe Pro Lys Ala Leu Ala
                85 90 95

Glu Ala Gly Ile Val Phe Ile Gly Pro Asn Pro Gly Ala Ile Ala Ala
            100 105 110

Met Gly Asp Lys Ile Glu Ser Lys Lys Ala Ala Ala Ala Ala Glu Val
        115 120 125

Ser Thr Val Pro Gly Phe Leu Gly Val Ile Glu Ser Pro Glu His Ala
    130 135 140

Val Thr Ile Ala Asp Glu Ile Gly Tyr Pro Val Met Ile Lys Ala Ser
145 150 155 160

Ala Gly Gly Gly Gly Lys Gly Met Arg Ile Ala Glu Ser Ala Asp Glu
                165 170 175

Val Ala Glu Gly Phe Ala Arg Ala Lys Ser Glu Ala Ser Ser Ser Phe
            180 185 190

Gly Asp Asp Arg Val Phe Val Glu Lys Phe Ile Thr Asp Pro Arg His
        195 200 205

Ile Glu Ile Gln Val Ile Gly Asp Lys His Gly Asn Val Ile Tyr Leu
    210 215 220

Gly Glu Arg Glu Cys Ser Ile Gln Arg Arg Asn Gln Lys Val Ile Glu
225 230 235 240

Glu Ala Pro Ser Pro Leu Leu Asp Glu Glu Thr Arg Arg Lys Met Gly
                245 250 255

Glu Gln Ala Val Ala Leu Ala Lys Ala Val Asn Tyr Asp Ser Ala Gly
            260 265 270

Thr Val Glu Phe Val Ala Gly Gln Asp Lys Ser Phe Tyr Phe Leu Glu
        275 280 285

Met Asn Thr Arg Leu Gln Val Glu His Pro Val Thr Glu Met Ile Thr
    290 295 300

Gly Leu Asp Leu Val Glu Leu Met Ile Arg Val Ala Ala Gly Glu Thr
305 310 315 320

Leu Pro Leu Thr Gln Asp Gln Val Lys Leu Asp Gly Trp Ala Val Glu
                325 330 335

Ser Arg Val Tyr Ala Glu Asp Pro Thr Arg Asn Phe Leu Pro Ser Ile

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            340                 345                 350

Gly Arg Leu Thr Thr Tyr Gln Pro Pro Glu Glu Gly Pro Leu Gly Gly
        355                 360                 365

Ala Ile Val Arg Asn Asp Thr Gly Val Glu Glu Gly Gly Glu Ile Ala
    370                 375                 380

Ile His Tyr Asp Pro Met Ile Ala Lys Leu Val Thr Trp Ala Pro Thr
385                 390                 395                 400

Arg Leu Glu Ala Ile Asp Ala Gln Ala Thr Ala Leu Asp Ala Phe Ala
                405                 410                 415

Ile Glu Gly Ile Arg His Asn Ile Pro Phe Leu Ala Thr Leu Met Ala
            420                 425                 430

His Pro Arg Trp Arg Asp Gly Arg Leu Ser Thr Gly Phe Ile Lys Glu
        435                 440                 445

Glu Phe Pro Glu Gly Phe Ile Ala Pro Glu Pro Glu Gly Pro Val Ala
    450                 455                 460

His Arg Leu Ala Ala Val Ala Ala Ala Ile Asp His Lys Leu Asn Ile
465                 470                 475                 480

Arg Lys Arg Gly Ile Ser Gly Gln Met Arg Asp Pro Ser Leu Leu Thr
                485                 490                 495

Phe Gln Arg Glu Arg Val Val Val Leu Ser Gly Gln Arg Phe Asn Val
            500                 505                 510

Thr Val Asp Pro Asp Gly Asp Asp Leu Leu Val Thr Phe Asp Asp Gly
        515                 520                 525

Thr Thr Ala Pro Val Arg Ser Ala Trp Arg Pro Gly Ala Pro Val Trp
    530                 535                 540

Ser Gly Thr Val Gly Asp Gln Ser Ile Ala Ile Gln Val Arg Pro Leu
545                 550                 555                 560

Leu Asn Gly Val Phe Leu Gln His Ala Gly Ala Ala Ala Glu Ala Arg
                565                 570                 575

Val Phe Thr Arg Arg Glu Ala Glu Leu Ala Asp Leu Met Pro Val Lys
            580                 585                 590

Glu Asn Ala Gly Ser Gly Lys Gln Leu Leu Cys Pro Met Pro Gly Leu
        595                 600                 605

Val Lys Gln Ile Met Val Ser Glu Gly Gln Glu Val Lys Asn Gly Glu
    610                 615                 620

Pro Leu Ala Ile Val Glu Ala Met Lys Met Glu Asn Val Leu Arg Ala
625                 630                 635                 640

Glu Arg Asp Gly Thr Ile Ser Lys Ile Ala Ala Lys Glu Gly Asp Ser
                645                 650                 655

Leu Ala Val Asp Ala Val Ile Leu Glu Phe Ala
            660                 665


<210> SEQ ID NO 56
<211> LENGTH: 510
<212> TYPE: PRT
<213> ORGANISM: Methylobacterium extorquens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_003065890
<309> DATABASE ENTRY DATE: 2010-04-16
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(510)

<400> SEQUENCE: 56

Met Lys Asp Ile Leu Glu Lys Leu Glu Glu Arg Arg Ala Gln Ala Arg
1               5                   10                  15
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Leu Gly Gly Gly Glu Lys Arg Leu Glu Ala Gln His Thr Arg Gly Lys
            20                  25                  30

Leu Thr Ala Arg Glu Arg Ile Glu Leu Leu Leu Asp His Gly Ser Phe
        35                  40                  45

Glu Glu Phe Asp Met Phe Val Gln His Arg Ser Thr Asp Phe Gly Met
    50                  55                  60

Glu Lys Gln Lys Ile Pro Gly Asp Gly Val Val Thr Gly Trp Gly Thr
65                  70                  75                  80

Val Asn Gly Arg Thr Val Phe Leu Phe Ser Lys Asp Phe Thr Val Phe
                85                  90                  95

Gly Gly Ser Leu Ser Glu Ala His Ala Ala Lys Ile Val Lys Val Gln
            100                 105                 110

Asp Met Ala Leu Lys Met Arg Ala Pro Ile Ile Gly Ile Phe Asp Ala
        115                 120                 125

Gly Gly Ala Arg Ile Gln Glu Gly Val Ala Ala Leu Gly Gly Tyr Gly
    130                 135                 140

Glu Val Phe Arg Arg Asn Val Ala Ala Ser Gly Val Ile Pro Gln Ile
145                 150                 155                 160

Ser Val Ile Met Gly Pro Cys Ala Gly Gly Asp Val Tyr Ser Pro Ala
                165                 170                 175

Met Thr Asp Phe Ile Phe Met Val Arg Asp Thr Ser Tyr Met Phe Val
            180                 185                 190

Thr Gly Pro Asp Val Val Lys Thr Val Thr Asn Glu Val Val Thr Ala
        195                 200                 205

Glu Glu Leu Gly Gly Ala Lys Val His Thr Ser Lys Ser Ser Ile Ala
    210                 215                 220

Asp Gly Ser Phe Glu Asn Asp Val Glu Ala Ile Leu Gln Ile Arg Arg
225                 230                 235                 240

Leu Leu Asp Phe Leu Pro Ala Asn Asn Ile Glu Gly Val Pro Glu Ile
                245                 250                 255

Glu Ser Phe Asp Asp Val Asn Arg Leu Asp Lys Ser Leu Asp Thr Leu
            260                 265                 270

Ile Pro Asp Asn Pro Asn Lys Pro Tyr Asp Met Gly Glu Leu Ile Arg
        275                 280                 285

Arg Val Val Asp Glu Gly Asp Phe Phe Glu Ile Gln Ala Ala Tyr Ala
    290                 295                 300

Arg Asn Ile Ile Thr Gly Phe Gly Arg Val Glu Gly Arg Thr Val Gly
305                 310                 315                 320

Phe Val Ala Asn Gln Pro Leu Val Leu Ala Gly Val Leu Asp Ser Asp
                325                 330                 335

Ala Ser Arg Lys Ala Ala Arg Phe Val Arg Phe Cys Asn Ala Phe Ser
            340                 345                 350

Ile Pro Ile Val Thr Phe Val Asp Val Pro Gly Phe Leu Pro Gly Thr
        355                 360                 365

Ala Gln Glu Tyr Gly Gly Leu Ile Lys His Gly Ala Lys Leu Leu Phe
    370                 375                 380

Ala Tyr Ser Gln Ala Thr Val Pro Leu Val Thr Ile Ile Thr Arg Lys
385                 390                 395                 400

Ala Phe Gly Gly Ala Tyr Asp Val Met Ala Ser Lys His Val Gly Ala
                405                 410                 415
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```
Asp Leu Asn Tyr Ala Trp Pro Thr Ala Gln Ile Ala Val Met Gly Ala
            420                 425                 430

Lys Gly Ala Val Glu Ile Ile Phe Arg Ala Glu Ile Gly Asp Ala Asp
        435                 440                 445

Lys Ile Ala Glu Arg Thr Lys Glu Tyr Glu Asp Arg Phe Leu Ser Pro
    450                 455                 460

Phe Val Ala Ala Glu Arg Gly Tyr Ile Asp Glu Val Ile Met Pro His
465                 470                 475                 480

Ser Thr Arg Lys Arg Ile Ala Arg Ala Leu Gly Met Leu Arg Thr Lys
                485                 490                 495

Glu Met Glu Gln Pro Trp Lys Lys His Asp Asn Ile Pro Leu
            500                 505                 510


<210> SEQ ID NO 57
<211> LENGTH: 670
<212> TYPE: PRT
<213> ORGANISM: Sinorhizobium meliloti
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / NP_437988
<309> DATABASE ENTRY DATE: 2010-04-01
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(670)

<400> SEQUENCE: 57

Met Gly His Met Phe Lys Lys Ile Leu Ile Ala Asn Arg Gly Glu Ile
1               5                   10                  15

Ala Cys Arg Val Ile Arg Thr Thr Lys Ala Leu Gly Ile Pro Thr Val
            20                  25                  30

Ala Val Tyr Ser Asp Ala Asp Arg Asp Ala Met His Val Arg Met Ala
        35                  40                  45

Asp Glu Ala Val His Ile Gly Pro Ser Pro Ser Ser Gln Ser Tyr Ile
    50                  55                  60

Val Ile Glu Asn Ile Leu Ala Ala Ile Arg Arg Thr Gly Ala Asp Ala
65                  70                  75                  80

Val His Pro Gly Tyr Gly Phe Leu Ser Glu Asn Ala Ala Phe Ala Glu
                85                  90                  95

Ala Leu Glu Lys Asp Gly Val Thr Phe Ile Gly Pro Pro Val Arg Ala
            100                 105                 110

Ile Glu Ala Met Gly Asp Lys Ile Thr Ser Lys Lys Leu Ala Ala Glu
        115                 120                 125

Ala Gly Val Phe Thr Val Pro Gly His Met Gly Leu Ile Glu Asp Ala
    130                 135                 140

Asp Glu Ala Ala Arg Ile Ala Ala Glu Ile Gly Phe Pro Val Met Ile
145                 150                 155                 160

Lys Ala Ser Ala Gly Gly Gly Gly Lys Gly Met Arg Ile Ala Trp Asn
                165                 170                 175

Glu Arg Glu Ala Arg Glu Gly Phe Gln Ser Ser Arg Asn Glu Ala Lys
            180                 185                 190

Ser Ser Phe Gly Asp Asp Arg Ile Phe Ile Glu Lys Phe Val Thr Glu
        195                 200                 205

Pro Arg His Ile Glu Ile Gln Val Leu Gly Asp Lys His Gly Asn Ile
    210                 215                 220

Leu Tyr Leu Gly Glu Arg Glu Cys Ser Ile Gln Arg Arg Asn Gln Lys
225                 230                 235                 240

Val Ile Glu Glu Ala Pro Ser Pro Phe Leu Asp Glu Lys Thr Arg Arg
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               245                 250                 255

Ala Met Gly Glu Gln Ala Val Ala Leu Ala Lys Ala Val Gly Tyr His
            260                 265                 270

Ser Ala Gly Thr Val Glu Phe Ile Val Asp Ala Gly Arg Asn Phe Tyr
        275                 280                 285

Phe Leu Glu Met Asn Thr Arg Leu Gln Val Glu His Pro Val Thr Glu
    290                 295                 300

Leu Val Thr Gly Leu Asp Leu Val Glu Gln Met Ile Arg Val Ala Ala
305                 310                 315                 320

Gly Ala Lys Leu Ala Phe Ala Gln Lys Asp Val Lys Leu Asp Gly Trp
                325                 330                 335

Ala Ile Glu Ser Arg Leu Tyr Ala Glu Asp Pro Tyr Arg Thr Phe Leu
            340                 345                 350

Pro Ser Ile Gly Arg Leu Thr Arg Tyr Arg Pro Pro Glu Glu Gly Thr
        355                 360                 365

Gln Ala Asp Gly Thr Val Ile Arg Asn Asp Thr Gly Val Phe Glu Gly
    370                 375                 380

Gly Glu Ile Ser Met Tyr Tyr Asp Pro Met Ile Ala Lys Leu Cys Thr
385                 390                 395                 400

Trp Gly Pro Asp Arg Leu Thr Ala Val Arg Ala Met Ala Asp Ala Leu
                405                 410                 415

Asp Ala Phe Glu Val Glu Gly Ile Gly His Asn Leu Pro Phe Leu Ala
            420                 425                 430

Ala Val Met Gln Gln Glu Arg Phe His Glu Gly Arg Leu Thr Thr Ala
        435                 440                 445

Tyr Ile Ala Glu Glu Phe Ala Gly Gly Phe His Gly Val Ala Leu Asp
    450                 455                 460

Asp Ala Ser Ala Arg Lys Leu Ala Ala Val Ala Ala Thr Val Asn Gln
465                 470                 475                 480

Thr Leu Gln Glu Arg Ala Ser Arg Ile Ser Gly Thr Ile Gly Asn His
                485                 490                 495

Arg Arg Val Val Gly His Glu Trp Val Thr Ser Leu Asp Gly His Glu
            500                 505                 510

Ile Gln Val Thr Cys Glu Val Ser Ala Asp Gly Thr Tyr Val Arg Phe
        515                 520                 525

Ala Asp Gly Thr Ser Val Ser Val Ala Thr Asp Trp Ala Pro Gly Arg
    530                 535                 540

Thr Arg Ala Ala Phe Asn Ile Asp Asn Gln Pro Met Ser Val Lys Val
545                 550                 555                 560

Glu Leu Ala Gly Pro Gly Ile Arg Leu Arg Trp Arg Gly Ile Asp Val
                565                 570                 575

Val Ala Arg Val Arg Ser Pro Arg Ile Ala Glu Leu Ala Arg Leu Met
            580                 585                 590

Pro Lys Lys Leu Pro Pro Asp Thr Ser Lys Met Leu Leu Cys Pro Met
        595                 600                 605

Pro Gly Val Val Thr Ser Ile Thr Val Lys Ala Gly Glu Thr Val Glu
    610                 615                 620

Ala Gly Gln Ala Ile Ala Val Val Glu Ala Met Lys Met Glu Asn Ile
625                 630                 635                 640

Leu Arg Ala Glu Lys Arg Ala Ile Val Lys Arg Val Ala Ile Glu Ala
                645                 650                 655
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Gly Ala Ser Leu Ala Val Asp Glu Leu Ile Met Glu Phe Glu
            660                 665                 670


<210> SEQ ID NO 58
<211> LENGTH: 510
<212> TYPE: PRT
<213> ORGANISM: Sinorhizobium meliloti
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / NP_437987
<309> DATABASE ENTRY DATE: 2010-04-01
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(510)

<400> SEQUENCE: 58

Met Arg Ala Val Leu Glu Gln Val Glu Ala Arg Arg Ala Glu Ala Arg
1               5                   10                  15

Ala Gly Gly Gly Glu Arg Arg Ile Ala Ala Gln His Gly Lys Gly Lys
            20                  25                  30

Leu Thr Ala Arg Glu Arg Ile Asp Val Leu Leu Asp Glu Gly Ser Phe
        35                  40                  45

Glu Glu Tyr Asp Met Tyr Val Thr His Arg Ser Val Asp Phe Gly Met
    50                  55                  60

Ala Gly Gln Lys Ile Pro Gly Asp Gly Val Val Thr Gly Trp Gly Thr
65                  70                  75                  80

Ile Asn Gly Arg Gln Val Tyr Val Phe Ser Gln Asp Phe Thr Val Leu
                85                  90                  95

Gly Gly Ser Leu Ser Glu Thr His Ala Gln Lys Ile Cys Lys Ile Met
            100                 105                 110

Asp Met Ala Ala Arg Asn Gly Ala Pro Val Ile Gly Leu Asn Asp Ser
        115                 120                 125

Gly Gly Ala Arg Ile Gln Glu Gly Val Ala Ser Leu Ala Gly Tyr Ala
    130                 135                 140

Glu Val Phe Arg Arg Asn Ala Glu Val Ser Gly Val Ile Pro Gln Ile
145                 150                 155                 160

Ser Val Ile Met Gly Pro Cys Ala Gly Gly Ala Val Tyr Ser Pro Ala
                165                 170                 175

Met Thr Asp Phe Ile Phe Met Val Arg Asp Ser Ser Tyr Met Phe Val
            180                 185                 190

Thr Gly Pro Asp Val Val Lys Thr Val Thr Asn Glu Ile Val Thr Ala
        195                 200                 205

Glu Glu Leu Gly Gly Ala Arg Thr His Thr Thr Lys Ser Ser Val Ala
    210                 215                 220

Asp Gly Ala Tyr Glu Asn Asp Ile Glu Ala Leu Glu His Val Arg Leu
225                 230                 235                 240

Leu Phe Asp Phe Leu Pro Leu Asn Asn Arg Glu Lys Pro Pro Val Arg
                245                 250                 255

Pro Phe His Asp Asp Pro Gly Arg Leu Glu Met Arg Leu Asp Ser Leu
            260                 265                 270

Ile Pro Asp Ser Ala Ala Lys Pro Tyr Asp Met Lys Glu Leu Ile Leu
        275                 280                 285

Ala Ile Ala Asp Glu Ala Asp Phe Phe Glu Leu Gln Ala Ser Phe Ala
    290                 295                 300

Arg Asn Ile Ile Thr Gly Phe Ile Arg Ile Glu Gly Gln Thr Val Gly
305                 310                 315                 320
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Val Ile Ala Asn Gln Pro Met Val Leu Ala Gly Cys Leu Asp Ile Asp
                325                 330                 335

Ser Ser Arg Lys Ala Ala Arg Phe Val Arg Phe Cys Asp Ala Phe Ser
            340                 345                 350

Ile Pro Ile Leu Thr Leu Val Asp Val Pro Gly Phe Leu Pro Gly Thr
        355                 360                 365

Ala Gln Glu Tyr Gly Gly Val Ile Lys His Gly Ala Lys Leu Leu Phe
    370                 375                 380

Ala Tyr Ser Gln Ala Thr Val Pro Met Val Thr Leu Ile Thr Arg Lys
385                 390                 395                 400

Ala Tyr Gly Gly Ala Tyr Asp Val Met Ala Ser Lys His Ile Gly Ala
                405                 410                 415

Asp Val Asn Tyr Ala Trp Pro Thr Ala Glu Ile Ala Val Met Gly Ala
            420                 425                 430

Lys Gly Ala Thr Glu Ile Leu Tyr Arg Ser Glu Leu Gly Asp Pro Ala
        435                 440                 445

Lys Ile Ala Ala Arg Thr Lys Glu Tyr Glu Glu Arg Phe Ala Asn Pro
    450                 455                 460

Phe Val Ala Ala Glu Arg Gly Phe Ile Asp Glu Val Ile Met Pro His
465                 470                 475                 480

Ser Ser Arg Arg Arg Ile Ala Arg Ala Phe Ala Ser Leu Arg Asn Lys
                485                 490                 495

Gln Val Glu Thr Arg Trp Arg Lys His Asp Thr Ile Pro Leu
            500                 505                 510


<210> SEQ ID NO 59
<211> LENGTH: 681
<212> TYPE: PRT
<213> ORGANISM: Ruegeria pomeroyi
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_166352
<309> DATABASE ENTRY DATE: 2010-06-29
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(681)

<400> SEQUENCE: 59

Met Phe Asn Lys Ile Leu Ile Ala Asn Arg Gly Glu Ile Ala Cys Arg
1               5                   10                  15

Val Ile Lys Thr Ala Arg Lys Met Gly Ile Ser Thr Val Ala Ile Tyr
            20                  25                  30

Ser Asp Ala Asp Lys Gln Ala Leu His Val Gln Met Ala Asp Glu Ala
        35                  40                  45

Val His Ile Gly Pro Pro Pro Ala Asn Gln Ser Tyr Ile Val Ile Asp
    50                  55                  60

Lys Val Met Ala Ala Ile Arg Ala Thr Gly Ala Gln Ala Val His Pro
65                  70                  75                  80

Gly Tyr Gly Phe Leu Ser Glu Asn Ser Lys Phe Ala Glu Ala Leu Glu
                85                  90                  95

Ala Glu Gly Val Ile Phe Val Gly Pro Pro Lys Gly Ala Ile Glu Ala
            100                 105                 110

Met Gly Asp Lys Ile Thr Ser Lys Lys Ile Ala Gln Glu Ala Asn Val
        115                 120                 125

Ser Thr Val Pro Gly Tyr Met Gly Leu Ile Glu Asp Ala Asp Glu Ala
    130                 135                 140

Val Lys Ile Ser Asn Gln Ile Gly Tyr Pro Val Met Ile Lys Ala Ser
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145                 150                 155                 160

Ala Gly Gly Gly Gly Lys Gly Met Arg Ile Ala Trp Asn Asp Gln Glu
                165                 170                 175

Ala Arg Glu Gly Phe Gln Ser Ser Lys Asn Glu Ala Ala Asn Ser Phe
            180                 185                 190

Gly Asp Asp Arg Ile Phe Ile Glu Lys Phe Val Thr Gln Pro Arg His
        195                 200                 205

Ile Glu Ile Gln Val Leu Cys Asp Ser His Gly Asn Gly Ile Tyr Leu
    210                 215                 220

Gly Glu Arg Glu Cys Ser Ile Gln Arg Arg Asn Gln Lys Val Val Glu
225                 230                 235                 240

Glu Ala Pro Ser Pro Phe Leu Asp Glu Ala Thr Arg Arg Ala Met Gly
                245                 250                 255

Glu Gln Ala Val Ala Leu Ala Lys Ala Val Gly Tyr Ala Ser Ala Gly
            260                 265                 270

Thr Val Glu Phe Ile Val Asp Gly Gln Lys Asn Phe Tyr Phe Leu Glu
        275                 280                 285

Met Asn Thr Arg Leu Gln Val Glu His Pro Val Thr Glu Leu Ile Thr
    290                 295                 300

Gly Val Asp Leu Val Glu Gln Met Ile Arg Val Ala Ala Gly Glu Pro
305                 310                 315                 320

Leu Ser Ile Thr Gln Gly Asp Val Lys Leu Thr Gly Trp Ala Ile Glu
                325                 330                 335

Asn Arg Leu Tyr Ala Glu Asp Pro Tyr Arg Gly Phe Leu Pro Ser Ile
            340                 345                 350

Gly Arg Leu Thr Arg Tyr Arg Pro Pro Ala Glu Thr Ala Ala Gly Pro
        355                 360                 365

Leu Leu Val Asn Gly Lys Trp Gln Gly Asp Ala Pro Ser Gly Glu Ala
    370                 375                 380

Ala Val Arg Asn Asp Thr Gly Val Tyr Glu Gly Gly Glu Ile Ser Met
385                 390                 395                 400

Tyr Tyr Asp Pro Met Ile Ala Lys Leu Cys Thr Trp Ala Pro Thr Arg
                405                 410                 415

Ala Ala Ala Ile Glu Ala Met Arg Ile Ala Leu Asp Ser Phe Glu Val
            420                 425                 430

Glu Gly Ile Gly His Asn Leu Pro Phe Leu Ser Ala Val Met Asp His
        435                 440                 445

Pro Lys Phe Ile Ser Gly Asp Met Thr Thr Ala Phe Ile Ala Glu Glu
    450                 455                 460

Tyr Pro Glu Gly Phe Glu Gly Val Asn Leu Pro Glu Thr Asp Leu Arg
465                 470                 475                 480

Arg Val Ala Ala Ala Ala Ala Ala Met His Arg Val Ala Glu Ile Arg
                485                 490                 495

Arg Thr Arg Val Ser Gly Arg Met Asp Asn His Glu Arg Arg Val Gly
            500                 505                 510

Thr Glu Trp Val Val Thr Leu Gln Gly Ala Asp Phe Pro Val Thr Ile
        515                 520                 525

Ala Ala Asp His Asp Gly Ser Thr Val Ser Phe Asp Asp Gly Ser Ser
    530                 535                 540

Met Arg Val Thr Ser Asp Trp Thr Pro Gly Asp Gln Leu Ala Asn Leu
545                 550                 555                 560
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Met Val Asp Gly Ala Pro Leu Val Leu Lys Val Gly Lys Ile Ser Gly
                565                 570                 575

Gly Phe Arg Ile Arg Thr Arg Gly Ala Asp Leu Lys Val His Val Arg
            580                 585                 590

Thr Pro Arg Gln Ala Glu Leu Ala Arg Leu Met Pro Glu Lys Leu Pro
        595                 600                 605

Pro Asp Thr Ser Lys Met Leu Leu Cys Pro Met Pro Gly Leu Ile Val
    610                 615                 620

Lys Val Asp Val Glu Val Gly Gln Glu Val Gln Glu Gly Gln Ala Leu
625                 630                 635                 640

Cys Thr Ile Glu Ala Met Lys Met Glu Asn Ile Leu Arg Ala Glu Lys
                645                 650                 655

Lys Gly Val Val Ala Lys Ile Asn Ala Ser Ala Gly Asn Ser Leu Ala
            660                 665                 670

Val Asp Asp Val Ile Met Glu Phe Glu
        675                 680


<210> SEQ ID NO 60
<211> LENGTH: 510
<212> TYPE: PRT
<213> ORGANISM: Ruegeria pomeroyi
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_166345
<309> DATABASE ENTRY DATE: 2010-06-29
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(510)

<400> SEQUENCE: 60

Met Lys Asp Ile Leu Ser Glu Leu Glu Thr Arg Arg Glu Ala Ala Arg
1               5                   10                  15

Leu Gly Gly Gly Gln Lys Arg Ile Asp Ala Gln His Ala Arg Gly Lys
            20                  25                  30

Leu Thr Ala Arg Glu Arg Ile Glu Leu Leu Leu Asp Glu Asp Ser Phe
        35                  40                  45

Glu Glu Phe Asp Met Phe Val Ser His Arg Cys Thr Asp Phe Gly Met
    50                  55                  60

Glu Lys Gln Arg Pro Ala Gly Asp Gly Val Val Thr Gly Trp Gly Thr
65                  70                  75                  80

Ile Asn Gly Arg Met Val Tyr Val Phe Ser Gln Asp Phe Thr Val Phe
                85                  90                  95

Gly Gly Ser Leu Ser Glu Thr His Ala Gln Lys Ile Cys Lys Ile Met
            100                 105                 110

Asp Met Ala Val Gln Asn Gly Ala Pro Val Ile Gly Ile Asn Asp Ser
        115                 120                 125

Gly Gly Ala Arg Ile Gln Glu Gly Val Ala Ser Leu Ala Gly Tyr Ala
    130                 135                 140

Glu Val Phe Gln Arg Asn Ile Met Ala Ser Gly Val Val Pro Gln Ile
145                 150                 155                 160

Ser Val Ile Met Gly Pro Cys Ala Gly Gly Ala Val Tyr Ser Pro Ala
                165                 170                 175

Met Thr Asp Phe Ile Phe Met Val Lys Asp Thr Ser Tyr Met Phe Val
            180                 185                 190

Thr Gly Pro Asp Val Val Lys Thr Val Thr Asn Glu Val Val Thr Ala
        195                 200                 205
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Glu Glu Leu Gly Gly Ala Ser Thr His Thr Arg Lys Ser Ser Val Ala
    210                 215                 220

Asp Gly Ala Phe Glu Asn Asp Val Glu Ala Leu Ala Glu Val Arg Arg
225                 230                 235                 240

Leu Val Asp Phe Leu Pro Leu Asn Asn Arg Glu Lys Pro Pro Val Arg
                245                 250                 255

Pro Phe Phe Asp Glu Pro Gly Arg Ile Glu Ala Ser Leu Asp Thr Leu
            260                 265                 270

Val Pro Glu Asn Ala Asn Thr Pro Tyr Asp Met Lys Glu Leu Ile Asn
        275                 280                 285

Lys Ile Ala Asp Glu Gly Asp Phe Tyr Glu Ile Gln Glu Asp Phe Ala
    290                 295                 300

Lys Asn Ile Ile Thr Gly Phe Ile Arg Leu Glu Gly Gln Thr Val Gly
305                 310                 315                 320

Val Val Ala Asn Gln Pro Met Ile Leu Ala Gly Cys Leu Asp Ile Asp
                325                 330                 335

Ser Ser Arg Lys Ala Ala Arg Phe Val Arg Phe Cys Asp Cys Phe Glu
            340                 345                 350

Ile Pro Ile Leu Thr Leu Val Asp Val Pro Gly Phe Leu Pro Gly Thr
        355                 360                 365

Ser Gln Glu Tyr Gly Gly Val Ile Lys His Gly Ala Lys Leu Leu Phe
    370                 375                 380

Ala Tyr Gly Glu Ala Thr Val Pro Lys Val Thr Val Ile Thr Arg Lys
385                 390                 395                 400

Ala Tyr Gly Gly Ala Tyr Asp Val Met Ala Ser Lys His Leu Arg Gly
                405                 410                 415

Asp Phe Asn Tyr Ala Trp Pro Thr Ala Glu Ile Ala Val Met Gly Ala
            420                 425                 430

Lys Gly Ala Thr Glu Ile Ile His Arg Ala Asp Leu Gly Asp Ala Asp
        435                 440                 445

Lys Ile Ala Ala His Thr Lys Asp Tyr Glu Gly Arg Phe Ala Asn Pro
    450                 455                 460

Phe Val Ala Ala Glu Arg Gly Phe Ile Asp Glu Val Ile Gln Pro Arg
465                 470                 475                 480

Ser Thr Arg Lys Arg Val Ser Arg Ala Phe Ala Ser Leu Arg Gly Lys
                485                 490                 495

Ser Leu Lys Asn Pro Trp Lys Lys His Asp Asn Ile Pro Leu
            500                 505                 510


<210> SEQ ID NO 61
<211> LENGTH: 678
<212> TYPE: PRT
<213> ORGANISM: Bacillus megaterium
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_003564880
<309> DATABASE ENTRY DATE: 2010-12-17
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(678)

<400> SEQUENCE: 61

Met Lys Thr Asn Thr Leu Ser Phe His Glu Phe Thr Arg Thr Pro Lys
1               5                   10                  15

Glu Asp Trp Ala Gln Glu Val Ser Lys Asn Thr Ala Ile Ser Ser Lys
            20                  25                  30

Glu Thr Leu Glu Asn Ile Phe Leu Lys Pro Leu Tyr Phe Glu Ser Asp
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        35                  40                  45

Thr Ala His Leu Asp Tyr Leu Gln Gln Ser Pro Ala Gly Ile Asp Tyr
    50                  55                  60

Leu Arg Gly Ala Gly Lys Glu Ser Tyr Ile Leu Gly Glu Trp Glu Ile
65                  70                  75                  80

Thr Gln Lys Ile Asp Leu Pro Ser Ile Lys Glu Ser Asn Lys Leu Leu
                85                  90                  95

Leu His Ser Leu Arg Asn Gly Gln Asn Thr Ala Ala Phe Thr Cys Ser
            100                 105                 110

Glu Ala Met Arg Gln Gly Lys Asp Ile Asp Glu Ala Thr Glu Ala Glu
        115                 120                 125

Val Ala Ser Gly Ala Thr Ile Ser Thr Leu Glu Asp Val Ala His Leu
    130                 135                 140

Phe Gln His Val Ala Leu Glu Ala Val Pro Leu Phe Leu Asn Thr Gly
145                 150                 155                 160

Cys Thr Ser Val Pro Leu Leu Ser Phe Leu Lys Ala Tyr Cys Val Asp
                165                 170                 175

His Asn Phe Asn Met Arg Gln Leu Lys Gly Thr Val Gly Met Asp Pro
            180                 185                 190

Leu Gly Thr Leu Ala Glu Tyr Gly Arg Val Pro Leu Ser Thr Arg Asp
        195                 200                 205

Leu Tyr Asp His Leu Ala Tyr Ala Thr Arg Leu Ala His Ser Asn Val
    210                 215                 220

Pro Glu Leu Lys Thr Ile Ile Val Ser Ser Ile Pro Tyr His Asn Ser
225                 230                 235                 240

Gly Ala Asn Ala Val Gln Glu Leu Ala Tyr Met Leu Ala Thr Gly Val
                245                 250                 255

Gln Tyr Ile Asp Glu Cys Ile Lys Arg Gly Leu Ser Leu His Gln Val
            260                 265                 270

Leu Pro His Met Thr Phe Ser Phe Ser Val Ser Ser His Leu Phe Met
        275                 280                 285

Glu Ile Ser Lys Leu Arg Ala Phe Arg Met Leu Trp Ala Asn Val Val
    290                 295                 300

Arg Ala Phe Asp Asp Thr Ala Val Ser Val Pro Phe Ile His Thr Glu
305                 310                 315                 320

Thr Ser His Leu Thr Gln Ser Lys Glu Asp Met Tyr Thr Asn Ala Leu
                325                 330                 335

Arg Ser Thr Val Gln Ala Phe Ala Ser Ile Val Gly Gly Ala Asp Ser
            340                 345                 350

Leu His Ile Glu Pro Tyr Asp Ser Val Thr Ser Ser Ser Ser Gln Phe
        355                 360                 365

Ala His Arg Leu Ala Arg Asn Thr His Leu Ile Leu Gln His Glu Thr
    370                 375                 380

His Ile Ser Lys Val Met Asp Pro Ala Gly Gly Ser Trp Tyr Val Glu
385                 390                 395                 400

Ala Tyr Thr His Glu Leu Met Thr Lys Ala Trp Glu Leu Phe Gly Asn
                405                 410                 415

Ile Glu Asp His Gly Gly Met Glu Glu Ala Leu Lys Gln Gly Arg Ile
            420                 425                 430

Gln Asp Glu Val Glu Gln Met Lys Val Lys Arg Gln Glu Asp Ile Glu
        435                 440                 445
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Cys Arg Ile Glu Arg Leu Ile Gly Val Thr His Tyr Ala Pro Lys Gln
    450                 455                 460

Gln Asp Ala Ser Gln Glu Ile Lys Ser Thr Pro Phe Lys Lys Glu Glu
465                 470                 475                 480

Ile Lys Met Asp Lys Tyr Ser Asp Gln Asn Ala Ser Glu Phe Ser Ser
                485                 490                 495

Asn Leu Ser Leu Glu Asp Tyr Thr Lys Leu Ala Ser Lys Gly Val Thr
            500                 505                 510

Ala Gly Trp Met Leu Lys Gln Met Ala Lys Gln Thr Gln Pro Asp Ser
        515                 520                 525

Val Val Pro Leu Thr Lys Trp Arg Ala Ala Glu Lys Phe Glu Lys Ile
    530                 535                 540

Arg Val Tyr Thr Lys Gly Met Ser Ile Gly Ile Met Glu Leu Thr Asp
545                 550                 555                 560

Pro Ser Ser Arg Lys Lys Ala Glu Ile Ala Arg Ser Leu Phe Glu Ser
                565                 570                 575

Ala Gly Phe Ala Cys Glu Thr Ile Lys Asn Ile Asp Ser Tyr Val Glu
            580                 585                 590

Ile Ala Asp Trp Met Asn Glu Gln Lys His Glu Ala Tyr Val Ile Cys
        595                 600                 605

Gly Ser Asp Glu Leu Val Glu Lys Leu Leu Thr Lys Ala Met Thr Tyr
    610                 615                 620

Phe Glu Glu Asp Ser Val Tyr Val Tyr Val Val Gly Glu Glu His Val
625                 630                 635                 640

Ser Arg Lys Thr Gln Trp Gln Gln Lys Gly Val Met Ser Val Ile His
                645                 650                 655

Pro Lys Thr Asn Val Ile Gln Cys Val Lys Lys Leu Leu Cys Ala Leu
            660                 665                 670

Glu Val Glu Val His Val
        675


<210> SEQ ID NO 62
<211> LENGTH: 716
<212> TYPE: PRT
<213> ORGANISM: Bacillus megaterium
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI - YP_003564879
<309> DATABASE ENTRY DATE: 2010-12-17
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(716)

<400> SEQUENCE: 62

Met Tyr Lys Lys Pro Ser Phe Ser Asn Ile Pro Leu Ser Phe Ser Lys
1               5                   10                  15

Gln Gln Arg Glu Asp Asp Val Thr Gln Ser Ser Tyr Thr Ala Phe Gln
            20                  25                  30

Thr Asn Glu Gln Ile Glu Leu Lys Ser Val Tyr Thr Lys Lys Asp Arg
        35                  40                  45

Asp Asn Leu Asp Phe Ile His Phe Ala Pro Gly Val Pro Pro Phe Val
    50                  55                  60

Arg Gly Pro Tyr Ala Thr Met Tyr Val Asn Arg Pro Trp Thr Ile Arg
65                  70                  75                  80

Gln Tyr Ala Gly Tyr Ser Thr Ala Glu Glu Ser Asn Ala Phe Tyr Arg
                85                  90                  95
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Arg Asn Leu Ala Ala Gly Gln Lys Gly Leu Ser Val Ala Phe Asp Leu
            100                 105                 110

Ala Thr His Arg Gly Tyr Asp Ser Asp His Pro Arg Val Val Gly Asp
        115                 120                 125

Val Gly Lys Ala Gly Val Ala Ile Asp Ser Met Met Asp Met Lys Gln
    130                 135                 140

Leu Phe Glu Gly Ile Pro Leu Asp Gln Met Ser Val Ser Met Thr Met
145                 150                 155                 160

Asn Gly Ala Val Leu Pro Ile Leu Ala Phe Tyr Ile Val Thr Ala Glu
                165                 170                 175

Glu Gln Gly Val Lys Lys Glu Lys Leu Ala Gly Thr Ile Gln Asn Asp
            180                 185                 190

Ile Leu Lys Glu Tyr Met Val Arg Asn Thr Tyr Ile Tyr Pro Pro Glu
        195                 200                 205

Met Ser Met Arg Ile Ile Ala Asp Ile Phe Lys Tyr Thr Ala Glu Tyr
    210                 215                 220

Met Pro Lys Phe Asn Ser Ile Ser Ile Ser Gly Tyr His Met Gln Glu
225                 230                 235                 240

Ala Gly Ala Pro Ala Asp Leu Glu Leu Ala Tyr Thr Leu Ala Asp Gly
                245                 250                 255

Leu Glu Tyr Val Arg Thr Gly Leu Lys Ala Gly Ile Thr Ile Asp Ala
            260                 265                 270

Phe Ala Pro Arg Leu Ser Phe Phe Trp Ala Ile Gly Met Asn Tyr Phe
        275                 280                 285

Met Glu Val Ala Lys Met Arg Ala Gly Arg Leu Leu Trp Ala Lys Leu
    290                 295                 300

Met Lys Gln Phe Glu Pro Asp Asn Pro Lys Ser Leu Ala Leu Arg Thr
305                 310                 315                 320

His Ser Gln Thr Ser Gly Trp Ser Leu Thr Glu Gln Asp Pro Phe Asn
                325                 330                 335

Asn Val Ile Arg Thr Cys Val Glu Ala Leu Ala Ala Val Ser Gly His
            340                 345                 350

Thr Gln Ser Leu His Thr Asn Ala Leu Asp Glu Ala Ile Ala Leu Pro
        355                 360                 365

Thr Asp Phe Ser Ala Arg Ile Ala Arg Asn Thr Gln Leu Tyr Leu Gln
    370                 375                 380

Asn Glu Thr Glu Ile Cys Ser Val Ile Asp Pro Trp Gly Gly Ser Tyr
385                 390                 395                 400

Tyr Val Glu Ser Leu Thr Asn Glu Leu Met Ile Lys Ala Trp Lys His
                405                 410                 415

Leu Glu Glu Ile Glu Gln Leu Gly Gly Met Thr Lys Ala Ile Glu Ala
            420                 425                 430

Gly Val Pro Lys Met Lys Ile Glu Glu Ala Ala Ala Arg Arg Gln Ala
        435                 440                 445

Arg Ile Asp Ser Gln Ala Glu Ile Ile Val Gly Val Asn Gln Phe Gln
    450                 455                 460

Pro Glu Gln Glu Glu Pro Leu Asp Ile Leu Asp Ile Asp Asn Thr Ala
465                 470                 475                 480

Val Arg Met Lys Gln Leu Glu Lys Leu Lys Lys Ile Arg Ser Glu Arg
                485                 490                 495

Asn Glu Gln Ala Val Ile Glu Ala Leu Asn Arg Leu Thr Asn Cys Ala
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           500                 505                 510

Lys Thr Gly Glu Gly Asn Leu Leu Ala Phe Ala Val Glu Ala Ala Arg
        515                 520                 525

Ala Arg Ala Thr Leu Gly Glu Ile Ser Glu Ala Ile Glu Lys Val Ala
    530                 535                 540

Gly Arg His Gln Ala Thr Ser Lys Ser Val Ser Gly Val Tyr Ser Ala
545                 550                 555                 560

Glu Phe Val His Arg Asp Gln Ile Glu Glu Val Arg Lys Leu Thr Ala
                565                 570                 575

Glu Phe Leu Glu Gly Glu Gly Arg Arg Pro Arg Ile Leu Val Ala Lys
            580                 585                 590

Met Gly Gln Asp Gly His Asp Arg Gly Ser Lys Val Ile Ser Thr Ala
        595                 600                 605

Phe Ala Asp Leu Gly Phe Asp Val Asp Ile Gly Pro Leu Phe Gln Thr
    610                 615                 620

Pro Gln Glu Thr Ala Arg Gln Ala Val Glu Asn Asp Val His Val Ile
625                 630                 635                 640

Gly Ile Ser Ser Leu Ala Ala Gly His Lys Thr Leu Leu Pro Gln Leu
                645                 650                 655

Val Asp Glu Leu Lys Lys Leu Glu Arg Asp Asp Ile Val Val Ile Val
            660                 665                 670

Gly Gly Val Ile Pro Lys Gln Asp Tyr Ser Phe Leu Leu Glu His Gly
        675                 680                 685

Ala Ser Ala Ile Phe Gly Pro Gly Thr Val Ile Pro Lys Ala Ala Val
    690                 695                 700

Ser Val Leu His Glu Ile Lys Lys Arg Leu Glu Glu
705                 710                 715


<210> SEQ ID NO 63
<211> LENGTH: 615
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_001282809
<309> DATABASE ENTRY DATE: 2010-05-13
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(615)

<400> SEQUENCE: 63

Met Ser Ile Asp Val Pro Glu Arg Ala Asp Leu Glu Gln Val Arg Gly
1               5                   10                  15

Arg Trp Arg Asn Ala Val Ala Gly Val Leu Ser Lys Ser Asn Arg Thr
            20                  25                  30

Asp Ser Ala Gln Leu Gly Asp His Pro Glu Arg Leu Leu Asp Thr Gln
        35                  40                  45

Thr Ala Asp Gly Phe Ala Ile Arg Ala Leu Tyr Thr Ala Phe Asp Glu
    50                  55                  60

Leu Pro Glu Pro Pro Leu Pro Gly Gln Trp Pro Phe Val Arg Gly Gly
65                  70                  75                  80

Asp Pro Leu Arg Asp Val His Ser Gly Trp Lys Val Ala Glu Ala Phe
                85                  90                  95

Pro Ala Asn Gly Ala Thr Ala Asp Thr Asn Ala Ala Val Leu Ala Ala
            100                 105                 110

Leu Gly Glu Gly Val Ser Ala Leu Leu Ile Arg Val Gly Glu Ser Gly
        115                 120                 125
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Val Ala Pro Asp Arg Leu Thr Ala Leu Leu Ser Gly Val Tyr Leu Asn
    130                 135                 140

Leu Ala Pro Val Ile Leu Asp Ala Gly Ala Asp Tyr Arg Pro Ala Cys
145                 150                 155                 160

Asp Val Met Leu Ala Leu Val Ala Gln Leu Asp Pro Gly Gln Arg Asp
                165                 170                 175

Thr Leu Ser Ile Asp Leu Gly Ala Asp Pro Leu Thr Ala Ser Leu Arg
            180                 185                 190

Asp Arg Pro Ala Pro Pro Ile Glu Glu Val Val Ala Val Ala Ser Arg
        195                 200                 205

Ala Ala Gly Glu Arg Gly Leu Arg Ala Ile Thr Val Asp Gly Pro Ala
    210                 215                 220

Phe His Asn Leu Gly Ala Thr Ala Ala Thr Glu Leu Ala Ala Thr Val
225                 230                 235                 240

Ala Ala Ala Val Ala Tyr Leu Arg Val Leu Thr Glu Ser Gly Leu Val
                245                 250                 255

Val Ser Asp Ala Leu Arg Gln Ile Ser Phe Arg Leu Ala Ala Asp Asp
            260                 265                 270

Asp Gln Phe Met Thr Leu Ala Lys Met Arg Ala Leu Arg Gln Leu Trp
        275                 280                 285

Ala Arg Val Ala Glu Val Val Gly Asp Pro Gly Gly Gly Ala Ala Val
    290                 295                 300

Val His Ala Glu Thr Ser Leu Pro Met Met Thr Gln Arg Asp Pro Trp
305                 310                 315                 320

Val Asn Met Leu Arg Cys Thr Leu Ala Ala Phe Gly Ala Gly Val Gly
                325                 330                 335

Gly Ala Asp Thr Val Leu Val His Pro Phe Asp Val Ala Ile Pro Gly
            340                 345                 350

Gly Phe Pro Gly Thr Ala Ala Gly Phe Ala Arg Arg Ile Ala Arg Asn
        355                 360                 365

Thr Gln Leu Leu Leu Leu Glu Glu Ser His Val Gly Arg Val Leu Asp
    370                 375                 380

Pro Ala Gly Gly Ser Trp Phe Val Glu Glu Leu Thr Asp Arg Leu Ala
385                 390                 395                 400

Arg Arg Ala Trp Gln Arg Phe Gln Ala Ile Glu Ala Arg Gly Gly Phe
                405                 410                 415

Val Glu Ala His Asp Phe Leu Ala Gly Gln Ile Ala Glu Cys Ala Ala
            420                 425                 430

Arg Arg Ala Asp Asp Ile Ala His Arg Arg Leu Ala Ile Thr Gly Val
        435                 440                 445

Asn Glu Tyr Pro Asn Leu Gly Glu Pro Ala Leu Pro Pro Gly Asp Pro
    450                 455                 460

Thr Ser Pro Val Arg Arg Tyr Ala Ala Gly Phe Glu Ala Leu Arg Asp
465                 470                 475                 480

Arg Ser Asp His His Leu Ala Arg Thr Gly Ala Arg Pro Arg Val Leu
                485                 490                 495

Leu Leu Pro Leu Gly Pro Leu Ala Glu His Asn Ile Arg Thr Thr Phe
            500                 505                 510

Ala Thr Asn Leu Leu Ala Ser Gly Gly Ile Glu Ala Ile Asp Pro Gly
        515                 520                 525
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Thr Val Asp Ala Gly Thr Val Gly Asn Ala Val Ala Asp Ala Gly Ser
    530                 535                 540

Pro Ser Val Ala Val Ile Cys Gly Thr Asp Ala Arg Tyr Arg Asp Glu
545                 550                 555                 560

Val Ala Asp Ile Val Gln Ala Ala Arg Ala Ala Gly Val Ser Arg Val
                565                 570                 575

Tyr Leu Ala Gly Pro Glu Lys Ala Leu Gly Asp Ala Ala His Arg Pro
            580                 585                 590

Asp Glu Phe Leu Thr Ala Lys Ile Asn Val Val Gln Ala Leu Ser Asn
        595                 600                 605

Leu Leu Thr Arg Leu Gly Ala
    610                 615


<210> SEQ ID NO 64
<211> LENGTH: 750
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_001282810
<309> DATABASE ENTRY DATE: 2010-05-13
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(750)

<400> SEQUENCE: 64

Met Thr Thr Lys Thr Pro Val Ile Gly Ser Phe Ala Gly Val Pro Leu
1               5                   10                  15

His Ser Glu Arg Ala Ala Gln Ser Pro Thr Glu Ala Ala Val His Thr
            20                  25                  30

His Val Ala Ala Ala Ala Ala Ala His Gly Tyr Thr Pro Glu Gln Leu
        35                  40                  45

Val Trp His Thr Pro Glu Gly Ile Asp Val Thr Pro Val Tyr Ile Ala
    50                  55                  60

Ala Asp Arg Ala Ala Ala Glu Ala Glu Gly Tyr Pro Leu His Ser Phe
65                  70                  75                  80

Pro Gly Glu Pro Pro Phe Val Arg Gly Pro Tyr Pro Thr Met Tyr Val
                85                  90                  95

Asn Gln Pro Trp Thr Ile Arg Gln Tyr Ala Gly Phe Ser Thr Ala Ala
            100                 105                 110

Asp Ser Asn Ala Phe Tyr Arg Arg Asn Leu Ala Ala Gly Gln Lys Gly
        115                 120                 125

Leu Ser Val Ala Phe Asp Leu Ala Thr His Arg Gly Tyr Asp Ser Asp
    130                 135                 140

His Pro Arg Val Gln Gly Asp Val Gly Met Ala Gly Val Ala Ile Asp
145                 150                 155                 160

Ser Ile Leu Asp Met Arg Gln Leu Phe Asp Gly Ile Asp Leu Ser Thr
                165                 170                 175

Val Ser Val Ser Met Thr Met Asn Gly Ala Val Leu Pro Ile Leu Ala
            180                 185                 190

Leu Tyr Val Val Ala Ala Glu Glu Gln Gly Val Ala Pro Glu Gln Leu
        195                 200                 205

Ala Gly Thr Ile Gln Asn Asp Ile Leu Lys Glu Phe Met Val Arg Asn
    210                 215                 220

Thr Tyr Ile Tyr Pro Pro Lys Pro Ser Met Arg Ile Ile Ser Asp Ile
225                 230                 235                 240

Phe Ala Tyr Thr Ser Ala Lys Met Pro Lys Phe Asn Ser Ile Ser Ile
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               245                 250                 255

Ser Gly Tyr His Ile Gln Glu Ala Gly Ala Thr Ala Asp Leu Glu Leu
            260                 265                 270

Ala Tyr Thr Leu Ala Asp Gly Val Asp Tyr Ile Arg Ala Gly Leu Asn
        275                 280                 285

Ala Gly Leu Asp Ile Asp Ser Phe Ala Pro Arg Leu Ser Phe Phe Trp
    290                 295                 300

Gly Ile Gly Met Asn Phe Phe Met Glu Val Ala Lys Leu Arg Ala Gly
305                 310                 315                 320

Arg Leu Leu Trp Ser Glu Leu Val Ala Gln Phe Ala Pro Lys Ser Ala
                325                 330                 335

Lys Ser Leu Ser Leu Arg Thr His Ser Gln Thr Ser Gly Trp Ser Leu
            340                 345                 350

Thr Ala Gln Asp Val Phe Asn Asn Val Ala Arg Thr Cys Ile Glu Ala
        355                 360                 365

Met Ala Ala Thr Gln Gly His Thr Gln Ser Leu His Thr Asn Ala Leu
    370                 375                 380

Asp Glu Ala Leu Ala Leu Pro Thr Asp Phe Ser Ala Arg Ile Ala Arg
385                 390                 395                 400

Asn Thr Gln Leu Val Leu Gln Gln Glu Ser Gly Thr Thr Arg Pro Ile
                405                 410                 415

Asp Pro Trp Gly Gly Ser Tyr Tyr Val Glu Trp Leu Thr His Arg Leu
            420                 425                 430

Ala Arg Arg Ala Arg Ala His Ile Ala Glu Val Ala Glu His Gly Gly
        435                 440                 445

Met Ala Gln Ala Ile Ser Asp Gly Ile Pro Lys Leu Arg Ile Glu Glu
    450                 455                 460

Ala Ala Ala Arg Thr Gln Ala Arg Ile Asp Ser Gly Gln Gln Pro Val
465                 470                 475                 480

Val Gly Val Asn Lys Tyr Gln Val Pro Glu Asp His Glu Ile Glu Val
                485                 490                 495

Leu Lys Val Glu Asn Ser Arg Val Arg Ala Glu Gln Leu Ala Lys Leu
            500                 505                 510

Gln Arg Leu Arg Ala Gly Arg Asp Glu Pro Ala Val Arg Ala Ala Leu
        515                 520                 525

Ala Glu Leu Thr Arg Ala Ala Ala Glu Gln Gly Arg Ala Gly Ala Asp
    530                 535                 540

Gly Leu Gly Asn Asn Leu Leu Ala Leu Ala Ile Asp Ala Ala Arg Ala
545                 550                 555                 560

Gln Ala Thr Val Gly Glu Ile Ser Glu Ala Leu Glu Lys Val Tyr Gly
                565                 570                 575

Arg His Arg Ala Glu Ile Arg Thr Ile Ser Gly Val Tyr Arg Asp Glu
            580                 585                 590

Val Gly Lys Ala Pro Asn Ile Ala Ala Ala Thr Glu Leu Val Glu Lys
        595                 600                 605

Phe Ala Glu Ala Asp Gly Arg Arg Pro Arg Ile Leu Ile Ala Lys Met
    610                 615                 620

Gly Gln Asp Gly His Asp Arg Gly Gln Lys Val Ile Ala Thr Ala Phe
625                 630                 635                 640

Ala Asp Ile Gly Phe Asp Val Asp Val Gly Ser Leu Phe Ser Thr Pro
                645                 650                 655
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Glu Glu Val Ala Arg Gln Ala Ala Asp Asn Asp Val His Val Ile Gly
            660                 665                 670

Val Ser Ser Leu Ala Ala Gly His Leu Thr Leu Val Pro Ala Leu Arg
        675                 680                 685

Asp Ala Leu Ala Gln Val Gly Arg Pro Asp Ile Met Ile Val Val Gly
    690                 695                 700

Gly Val Ile Pro Pro Gly Asp Phe Asp Glu Leu Tyr Ala Ala Gly Ala
705                 710                 715                 720

Thr Ala Ile Phe Pro Pro Gly Thr Val Ile Ala Asp Ala Ala Ile Asp
                725                 730                 735

Leu Leu His Arg Leu Ala Glu Arg Leu Gly Tyr Thr Leu Asp
            740                 745                 750


<210> SEQ ID NO 65
<211> LENGTH: 616
<212> TYPE: PRT
<213> ORGANISM: Corynebacterium glutamicum
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_225814
<309> DATABASE ENTRY DATE: 2010-12-14
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(616)

<400> SEQUENCE: 65

Met Thr Asp Leu Thr Lys Thr Ala Val Pro Glu Glu Leu Ser Glu Asn
1               5                   10                  15

Leu Glu Thr Trp Tyr Lys Ala Val Ala Gly Val Phe Ala Arg Thr Gln
            20                  25                  30

Lys Lys Asp Ile Gly Asp Ile Ala Val Asp Val Trp Lys Lys Leu Ile
        35                  40                  45

Val Thr Thr Pro Asp Gly Val Asp Ile Asn Pro Leu Tyr Thr Arg Ala
    50                  55                  60

Asp Glu Ser Gln Arg Lys Phe Thr Glu Val Pro Gly Glu Phe Pro Phe
65                  70                  75                  80

Thr Arg Gly Thr Thr Val Asp Gly Glu Arg Val Gly Trp Gly Val Thr
                85                  90                  95

Glu Thr Phe Gly His Asp Ser Pro Lys Asn Ile Asn Ala Ala Val Leu
            100                 105                 110

Asn Ala Leu Asn Ser Gly Thr Thr Thr Leu Gly Phe Glu Phe Ser Glu
        115                 120                 125

Glu Phe Thr Ala Ala Asp Leu Lys Val Ala Leu Glu Gly Val Tyr Leu
    130                 135                 140

Asn Met Ala Pro Leu Leu Ile His Ala Gly Gly Ser Thr Ser Glu Val
145                 150                 155                 160

Ala Ala Ala Leu Tyr Thr Leu Ala Glu Glu Ala Gly Thr Phe Phe Ala
                165                 170                 175

Ala Leu Thr Leu Gly Ser Arg Pro Leu Thr Ala Gln Val Asp Gly Ser
            180                 185                 190

His Ser Asp Thr Ile Glu Glu Ala Val Gln Leu Ala Val Asn Ala Ser
        195                 200                 205

Lys Arg Ala Asn Val Arg Ala Ile Leu Val Asp Gly Ser Ser Phe Ser
    210                 215                 220

Asn Gln Gly Ala Ser Asp Ala Gln Glu Ile Gly Leu Ser Ile Ala Ala
225                 230                 235                 240
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```
Gly Val Asp Tyr Val Arg Arg Leu Val Asp Ala Gly Leu Ser Thr Glu
                245                 250                 255

Ala Ala Leu Lys Gln Val Ala Phe Arg Phe Ala Val Thr Asp Glu Gln
            260                 265                 270

Phe Ala Gln Ile Ser Lys Leu Arg Val Ala Arg Arg Leu Trp Ala Arg
        275                 280                 285

Val Cys Glu Val Leu Gly Phe Pro Glu Leu Ala Val Ala Pro Gln His
    290                 295                 300

Ala Val Thr Ala Arg Ala Met Phe Ser Gln Arg Asp Pro Trp Val Asn
305                 310                 315                 320

Met Leu Arg Ser Thr Val Ala Ala Phe Ala Ala Gly Val Gly Gly Ala
                325                 330                 335

Thr Asp Val Glu Val Arg Thr Phe Asp Asp Ala Ile Pro Asp Gly Val
            340                 345                 350

Pro Gly Val Ser Arg Asn Phe Ala His Arg Ile Ala Arg Asn Thr Asn
        355                 360                 365

Leu Leu Leu Leu Glu Glu Ser His Leu Gly His Val Val Asp Pro Ala
    370                 375                 380

Gly Gly Ser Tyr Phe Val Glu Ser Phe Thr Asp Asp Leu Ala Glu Lys
385                 390                 395                 400

Ala Trp Ala Val Phe Ser Gly Ile Glu Ala Glu Gly Gly Tyr Ser Ala
                405                 410                 415

Ala Cys Ala Ser Gly Thr Val Thr Ala Met Leu Asp Gln Thr Trp Glu
            420                 425                 430

Gln Thr Arg Ala Asp Val Ala Ser Arg Lys Lys Lys Leu Thr Gly Ile
        435                 440                 445

Asn Glu Phe Pro Asn Leu Ala Glu Ser Pro Leu Pro Ala Asp Arg Arg
    450                 455                 460

Val Glu Pro Ala Gly Val Arg Arg Trp Ala Ala Asp Phe Glu Ala Leu
465                 470                 475                 480

Arg Asn Arg Ser Asp Ala Phe Leu Glu Lys Asn Gly Ala Arg Pro Gln
                485                 490                 495

Ile Thr Met Ile Pro Leu Gly Pro Leu Ser Lys His Asn Ile Arg Thr
            500                 505                 510

Gly Phe Thr Ser Asn Leu Leu Ala Ser Gly Gly Ile Glu Ala Ile Asn
        515                 520                 525

Pro Gly Gln Leu Val Pro Gly Thr Asp Ala Phe Ala Glu Ala Ala Gln
    530                 535                 540

Ala Ala Gly Ile Val Val Val Cys Gly Thr Asp Gln Glu Tyr Ala Glu
545                 550                 555                 560

Thr Gly Glu Gly Ala Val Glu Lys Leu Arg Glu Ala Gly Val Glu Arg
                565                 570                 575

Ile Leu Leu Ala Gly Ala Pro Lys Ser Phe Glu Gly Ser Ala His Ala
            580                 585                 590

Pro Asp Gly Tyr Leu Asn Met Thr Ile Asp Ala Ala Ala Thr Leu Ala
        595                 600                 605

Asp Leu Leu Asp Ala Leu Gly Ala
    610                 615


<210> SEQ ID NO 66
<211> LENGTH: 737
<212> TYPE: PRT
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<213> ORGANISM: Corynebacterium glutamicum
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_225813
<309> DATABASE ENTRY DATE: 2010-12-14
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(737)

<400> SEQUENCE: 66

```
Met Thr Ser Ile Pro Asn Phe Ser Asp Ile Pro Leu Thr Ala Glu Thr
1               5                  10                  15

Arg Ala Ser Glu Ser His Asn Val Asp Ala Gly Lys Val Trp Asn Thr
            20                  25                  30

Pro Glu Gly Ile Asp Val Lys Arg Val Phe Thr Gln Ala Asp Arg Asp
        35                  40                  45

Glu Ala Gln Ala Ala Gly His Pro Val Asp Ser Leu Pro Gly Gln Lys
    50                  55                  60

Pro Phe Met Arg Gly Pro Tyr Pro Thr Met Tyr Thr Asn Gln Pro Trp
65                  70                  75                  80

Thr Ile Arg Gln Tyr Ala Gly Phe Ser Thr Ala Ala Glu Ser Asn Ala
                85                  90                  95

Phe Tyr Arg Arg Asn Leu Ala Ala Gly Gln Lys Gly Leu Ser Val Ala
            100                 105                 110

Phe Asp Leu Ala Thr His Arg Gly Tyr Asp Ser Asp Asn Glu Arg Val
        115                 120                 125

Val Gly Asp Val Gly Met Ala Gly Val Ala Ile Asp Ser Ile Leu Asp
    130                 135                 140

Met Arg Gln Leu Phe Asp Gly Ile Asp Leu Ser Ser Val Ser Val Ser
145                 150                 155                 160

Met Thr Met Asn Gly Ala Val Leu Pro Ile Leu Ala Phe Tyr Ile Val
                165                 170                 175

Ala Ala Glu Glu Gln Gly Val Gly Pro Glu Gln Leu Ala Gly Thr Ile
            180                 185                 190

Gln Asn Asp Ile Leu Lys Glu Phe Met Val Arg Asn Thr Tyr Ile Tyr
        195                 200                 205

Pro Pro Lys Pro Ser Met Arg Ile Ile Ser Asn Ile Phe Glu Tyr Thr
    210                 215                 220

Ser Leu Lys Met Pro Arg Phe Asn Ser Ile Ser Ile Ser Gly Tyr His
225                 230                 235                 240

Ile Gln Glu Ala Gly Ala Thr Ala Asp Leu Glu Leu Ala Tyr Thr Leu
                245                 250                 255

Ala Asp Gly Ile Glu Tyr Ile Arg Ala Gly Lys Glu Val Gly Leu Asp
            260                 265                 270

Val Asp Lys Phe Ala Pro Arg Leu Ser Phe Phe Trp Gly Ile Ser Met
        275                 280                 285

Tyr Thr Phe Met Glu Ile Ala Lys Leu Arg Ala Gly Arg Leu Leu Trp
    290                 295                 300

Ser Glu Leu Val Ala Lys Phe Asp Pro Lys Asn Ala Lys Ser Gln Ser
305                 310                 315                 320

Leu Arg Thr His Ser Gln Thr Ser Gly Trp Ser Leu Thr Ala Gln Asp
                325                 330                 335

Val Tyr Asn Asn Val Ala Arg Thr Ala Ile Glu Ala Met Ala Ala Thr
            340                 345                 350

Gln Gly His Thr Gln Ser Leu His Thr Asn Ala Leu Asp Glu Ala Leu
        355                 360                 365
```

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```
Ala Leu Pro Thr Asp Phe Ser Ala Arg Ile Ala Arg Asn Thr Gln Leu
    370                 375                 380

Leu Leu Gln Gln Glu Ser Gly Thr Val Arg Pro Val Asp Pro Trp Ala
385                 390                 395                 400

Gly Ser Tyr Tyr Val Glu Trp Leu Thr Asn Glu Leu Ala Asn Arg Ala
                405                 410                 415

Arg Lys His Ile Asp Glu Val Glu Glu Ala Gly Gly Met Ala Gln Ala
            420                 425                 430

Thr Ala Gln Gly Ile Pro Lys Leu Arg Ile Glu Glu Ser Ala Ala Arg
        435                 440                 445

Thr Gln Ala Arg Ile Asp Ser Gly Arg Gln Ala Leu Ile Gly Val Asn
    450                 455                 460

Arg Tyr Val Ala Glu Glu Asp Glu Glu Ile Glu Val Leu Lys Val Asp
465                 470                 475                 480

Asn Thr Lys Val Arg Ala Glu Gln Leu Ala Lys Leu Ala Gln Leu Lys
                485                 490                 495

Ala Glu Arg Asn Asp Ala Glu Val Lys Ala Ala Leu Asp Ala Leu Thr
            500                 505                 510

Ala Ala Ala Arg Asn Glu His Lys Glu Pro Gly Asp Leu Asp Gln Asn
        515                 520                 525

Leu Leu Lys Leu Ala Val Asp Ala Ala Arg Ala Lys Ala Thr Ile Gly
    530                 535                 540

Glu Ile Ser Asp Ala Leu Glu Val Val Phe Gly Arg His Glu Ala Glu
545                 550                 555                 560

Ile Arg Thr Leu Ser Gly Val Tyr Lys Asp Glu Val Gly Lys Glu Gly
                565                 570                 575

Thr Val Ser Asn Val Glu Arg Ala Ile Ala Leu Ala Asp Ala Phe Glu
            580                 585                 590

Ala Glu Glu Gly Arg Arg Pro Arg Ile Phe Ile Ala Lys Met Gly Gln
        595                 600                 605

Asp Gly His Asp Arg Gly Gln Lys Val Val Ala Ser Ala Tyr Ala Asp
    610                 615                 620

Leu Gly Met Asp Val Asp Val Gly Pro Leu Phe Gln Thr Pro Ala Glu
625                 630                 635                 640

Ala Ala Arg Ala Ala Val Asp Ala Asp Val His Val Val Gly Met Ser
                645                 650                 655

Ser Leu Ala Ala Gly His Leu Thr Leu Leu Pro Glu Leu Lys Lys Glu
            660                 665                 670

Leu Ala Ala Leu Gly Arg Asp Asp Ile Leu Val Thr Val Gly Gly Val
        675                 680                 685

Ile Pro Pro Gly Asp Phe Gln Asp Leu Tyr Asp Met Gly Ala Ala Ala
    690                 695                 700

Ile Tyr Pro Pro Gly Thr Val Ile Ala Glu Ser Ala Ile Asp Leu Ile
705                 710                 715                 720

Thr Arg Leu Ala Ala His Leu Gly Phe Asp Leu Asp Val Asp Val Asn
                725                 730                 735

Glu
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<210> SEQ ID NO 67
<211> LENGTH: 631
<212> TYPE: PRT

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<213> ORGANISM: Rhodococcus erythropolis
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_002766535
<309> DATABASE ENTRY DATE: 2010-05-12
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(631)

<400> SEQUENCE: 67

```
Met Ser Leu Ala Ser Glu Ala Glu Ala Val Glu Gln Ala Tyr Ala Glu
1               5                  10                  15

Trp Gln Arg Ser Val Ala Gly Val Leu Ala Lys Ser Arg Arg Val Asp
            20                  25                  30

Ala Ala Glu Leu Gly Pro Glu Pro Gln Lys Leu Leu Glu Thr Val Thr
        35                  40                  45

Tyr Asp Gly Val Thr Val Ala Pro Leu Tyr Ser Pro Arg Asp Glu Arg
    50                  55                  60

Pro Glu Gln Ser Leu Pro Gly Thr Phe Pro Tyr Val Arg Gly Val Asp
65                  70                  75                  80

Ala His Arg Asp Val Asn Ala Gly Trp Leu Val Ser Ala Ala Phe Gly
                85                  90                  95

Thr Ala Ser Ala Ala Glu Thr Asn Arg Ala Ile Leu Asp Ala Leu Glu
            100                 105                 110

Asn Gly Val Ser Ala Leu Trp Leu Lys Val Gly Ala Asp Gly Val Pro
        115                 120                 125

Val Thr Asp Leu Ala Ala Ala Leu Glu Gly Val Leu Leu Asp Leu Ala
    130                 135                 140

Pro Leu Thr Leu Asp Ala Gly Ala Glu Val Asn Asp Ala Ala Arg Ala
145                 150                 155                 160

Leu Phe Ser Leu Leu Asp Ala Arg Gly Glu Ala Gly Asp Gly Val Ser
                165                 170                 175

Asp Arg Ser Ser Ile Arg Val His Leu Gly Ala Ala Pro Leu Thr Ser
            180                 185                 190

Ser Phe Ser Gly Ala Ala Asp Val Glu Phe Ala Gly Ala Val Glu Leu
        195                 200                 205

Ala Ala Leu Ala Ala Ala Arg Ala Glu Thr Val His Ala Ile Thr Val
    210                 215                 220

Asp Gly Thr Ala Phe His Asn Ala Gly Ala Gly Asp Ala Glu Glu Leu
225                 230                 235                 240

Gly Ala Ala Ile Ala Ala Gly Leu Glu Tyr Leu Arg Ala Leu Thr Ala
                245                 250                 255

Glu Ser Gly Leu Thr Ile Gly Ala Ala Leu Ser Gln Leu Ala Phe Arg
            260                 265                 270

Tyr Ser Ala Thr Asp Asp Gln Phe Gln Thr Ile Ala Lys Phe Arg Ala
        275                 280                 285

Ala Arg Leu Val Trp Ala Arg Ile Ala Gln Val Cys Gly Ala Ser Asp
    290                 295                 300

Phe Gly Gly Ala Pro Gln His Ala Val Thr Ser Ala Ala Met Met Ala
305                 310                 315                 320

Gln Arg Asp Pro Trp Val Asn Met Leu Arg Thr Thr Leu Ala Ala Phe
                325                 330                 335

Gly Ala Gly Val Gly Gly Ala Asp Ala Val Thr Val Leu Pro Phe Asp
            340                 345                 350

Val Ala Leu Ala Asp Gly Thr Leu Gly Val Ser Lys Ser Phe Ser Ser
        355                 360                 365
```

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```
Arg Ile Ala Arg Asn Thr Gln Leu Leu Leu Leu Glu Glu Ser His Leu
    370                 375                 380

Gly Arg Val Leu Asp Pro Ser Ala Gly Ser Trp Tyr Val Glu Asp Leu
385                 390                 395                 400

Thr Gln Gln Ile Ala Ala Thr Ala Trp Glu Phe Phe Gln Glu Ile Glu
                405                 410                 415

Ala Ala Gly Gly Tyr Leu Ala Ala Leu Glu Ala Gly Ile Val Ser Gly
            420                 425                 430

Arg Ile Ala Ala Thr Lys Ala Lys Arg Asp Ser Asp Ile Ala His Arg
        435                 440                 445

Lys Thr Thr Val Thr Gly Val Asn Glu Phe Pro Asn Leu Gly Glu Thr
    450                 455                 460

Pro Leu Ser Ala Glu Ala Val Glu Pro Gly Gln Ser Val Ala Arg Tyr
465                 470                 475                 480

Ala Ala Ala Phe Glu Ala Leu Arg Asp Arg Ser Asp Ala Phe Leu Ala
                485                 490                 495

Ala Gly Gly Ala Arg Pro Thr Ala Leu Leu Ala Pro Leu Gly Ser Val
            500                 505                 510

Ala Glu His Asn Val Arg Thr Thr Phe Ala Ser Asn Leu Leu Ala Ser
        515                 520                 525

Gly Gly Ile Asp Ala Val Asn Pro Gly Pro Leu Glu Val Gly Ala Glu
    530                 535                 540

Ala Ile Ser Ala Ala Val Lys Ala Ser Gly Val Thr Val Ala Val Leu
545                 550                 555                 560

Cys Gly Thr Asp Lys Arg Tyr Gly Glu Ser Ala Ala Ala Ala Val Ala
                565                 570                 575

Glu Leu Arg Ala Ala Gly Ile Thr Lys Val Leu Leu Ala Gly Pro Glu
            580                 585                 590

Lys Ala Val Ala Asp Ala Thr Gly Glu Ser Arg Pro Asp Gly Phe Leu
        595                 600                 605

Thr Ala Arg Ile Asp Ala Val Ser Ala Leu Thr Glu Leu Leu Asp Phe
    610                 615                 620

Ile Glu Thr Gly Ser Ser Lys
625                 630


<210> SEQ ID NO 68
<211> LENGTH: 750
<212> TYPE: PRT
<213> ORGANISM: Rhodococcus erythropolis
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / YP_002766536
<309> DATABASE ENTRY DATE: 2010-05-12
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(750)

<400> SEQUENCE: 68

Met Thr Thr Arg Glu Val Lys His Val Ile Gly Ser Phe Ala Glu Val
1               5                   10                  15

Pro Leu Glu Asp Pro Gln Ser Pro Ala Pro Thr Pro Pro Ser Val Glu
            20                  25                  30

Gln Ala Gln Ala Leu Ile Glu Glu Gly Ala Asn Ala Asn Asn Tyr Ala
        35                  40                  45

Ala Glu Gln Val Val Trp Ser Thr Pro Glu Gly Ile Asp Val Lys Pro
    50                  55                  60
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```
Val Tyr Thr Gly Ala Asp Arg Thr Ala Ala Ala Glu Ser Gly Tyr Pro
65                  70                  75                  80

Leu Asp Ser Phe Pro Gly Ala Ala Pro Phe Leu Arg Gly Pro Tyr Pro
                85                  90                  95

Thr Met Tyr Val Asn Gln Pro Trp Thr Ile Arg Gln Tyr Ala Gly Phe
            100                 105                 110

Ser Thr Ala Ala Glu Ser Asn Ala Phe Tyr Arg Arg Asn Leu Ala Ala
        115                 120                 125

Gly Gln Lys Gly Leu Ser Val Ala Phe Asp Leu Ala Thr His Arg Gly
    130                 135                 140

Tyr Asp Ser Asp His Pro Arg Val Ala Gly Asp Val Gly Met Ala Gly
145                 150                 155                 160

Val Ala Ile Asp Ser Ile Leu Asp Met Arg Gln Leu Phe Asp Gly Ile
                165                 170                 175

Asp Leu Ser Gln Val Ser Val Ser Met Thr Met Asn Gly Ala Val Leu
            180                 185                 190

Pro Ile Leu Ala Leu Tyr Val Ala Ala Ala Gly Glu Gln Gly Val Thr
        195                 200                 205

Pro Asp Lys Leu Ala Gly Thr Ile Gln Asn Asp Ile Leu Lys Glu Phe
    210                 215                 220

Met Val Arg Asn Thr Tyr Ile Tyr Pro Pro Lys Pro Ser Met Arg Ile
225                 230                 235                 240

Ile Ser Asp Ile Phe Ala Tyr Ser Ser Ala Glu Met Pro Lys Tyr Asn
                245                 250                 255

Ser Ile Ser Ile Ser Gly Tyr His Ile Gln Glu Ala Gly Ala Thr Ala
            260                 265                 270

Asp Leu Glu Leu Ala Tyr Thr Leu Ala Asp Gly Val Glu Tyr Ile Arg
        275                 280                 285

Ala Gly Leu Asp Ala Gly Met Asp Ile Asp Lys Phe Ala Pro Arg Leu
    290                 295                 300

Ser Phe Phe Trp Ala Ile Gly Met Asn Phe Phe Met Glu Val Ala Lys
305                 310                 315                 320

Leu Arg Ala Gly Arg Leu Leu Trp Ala Glu Leu Val Ala Lys Phe Asp
                325                 330                 335

Pro Lys Ser Ala Lys Ser Leu Ser Leu Arg Thr His Ser Gln Thr Ser
            340                 345                 350

Gly Trp Ser Leu Thr Ala Gln Asp Val Phe Asn Asn Val Pro Arg Thr
        355                 360                 365

Cys Val Glu Ala Met Ala Ala Thr Gln Gly His Thr Gln Ser Leu His
    370                 375                 380

Thr Asn Ala Leu Asp Glu Ala Ile Ala Leu Pro Thr Asp Phe Ser Ala
385                 390                 395                 400

Arg Ile Ala Arg Asn Thr Gln Leu Leu Leu Gln Gln Glu Ser Gly Thr
                405                 410                 415

Val Arg Pro Ile Asp Pro Trp Gly Gly Ser Tyr Tyr Val Glu Trp Leu
            420                 425                 430

Thr Asn Glu Leu Ala Asn Arg Ala Arg Lys His Ile Glu Glu Val Glu
        435                 440                 445

Glu Ala Gly Gly Met Ala Gln Ala Ile Asn Glu Gly Ile Pro Lys Leu
    450                 455                 460

Arg Ile Glu Glu Ala Ala Ala Arg Thr Gln Ala Arg Ile Asp Ser Gly
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```
465                 470                 475                 480

Arg Gln Pro Leu Val Gly Val Asn Lys Tyr Val Pro Asp Glu Val Asp
                485                 490                 495

Thr Ile Glu Val Leu Lys Val Glu Asn Ser Lys Val Arg Lys Glu Gln
            500                 505                 510

Leu Glu Lys Leu Val Arg Leu Arg Ala Glu Arg Asp Pro Glu Ala Val
        515                 520                 525

Glu Ala Ala Leu Ala Asn Leu Thr Arg Ala Ala Ala Ser Thr Glu Gly
    530                 535                 540

Gly Met Glu Asn Asn Leu Leu Ala Leu Ala Val Val Ala Ala Arg Ala
545                 550                 555                 560

Met Ala Thr Val Gly Glu Ile Ser Asp Ala Leu Glu Lys Val Tyr Gly
                565                 570                 575

Arg His Gln Ala Glu Ile Arg Thr Ile Ser Gly Val Tyr Arg Asp Glu
            580                 585                 590

Ala Gly Thr Val Ser Asn Ile Ser Lys Ala Met Glu Leu Val Glu Lys
        595                 600                 605

Phe Ala Glu Asp Glu Gly Arg Arg Pro Arg Ile Leu Val Ala Lys Met
    610                 615                 620

Gly Gln Asp Gly His Asp Arg Gly Gln Lys Val Ile Ser Thr Ala Phe
625                 630                 635                 640

Ala Asp Ile Gly Phe Asp Val Asp Val Gly Pro Leu Phe Gln Thr Pro
                645                 650                 655

Glu Glu Val Ala Asn Gln Ala Ala Asp Asn Asp Val His Val Val Gly
            660                 665                 670

Val Ser Ser Leu Ala Ala Gly His Leu Thr Leu Val Pro Ala Leu Arg
        675                 680                 685

Glu Ala Leu Ala Ala Ala Gly Arg Pro Asp Ile Met Ile Val Val Gly
    690                 695                 700

Gly Val Ile Pro Pro Gly Asp Phe Asp Glu Leu Tyr Glu Ala Gly Ala
705                 710                 715                 720

Ala Ala Ile Phe Pro Pro Gly Thr Val Ile Ala Asp Ala Ala Ser Gly
                725                 730                 735

Leu Leu Glu Lys Leu Ser Ala Gln Leu Gly His Asp His Ser
            740                 745                 750


<210> SEQ ID NO 69
<211> LENGTH: 618
<212> TYPE: PRT
<213> ORGANISM: Porphyromonas gingivalis
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / NP_905776
<309> DATABASE ENTRY DATE: 2010-06-29
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(618)

<400> SEQUENCE: 69

Met Ala Lys Glu Lys Glu Lys Leu Phe Ser Glu Phe Pro Pro Val Ser
1                 5                  10                  15

Arg Glu Ala Trp Ile Asp Lys Ile Thr Ala Asp Leu Lys Gly Val Pro
            20                  25                  30

Phe Glu Lys Lys Leu Val Trp Arg Thr Asn Glu Gly Phe Asn Val Asn
        35                  40                  45

Pro Phe Tyr Arg Arg Glu Asp Ile Glu Asp Leu Lys Thr Thr Thr Ser
    50                  55                  60
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Leu Pro Asp Glu Tyr Pro Tyr Val Arg Ser Thr Arg Met His Asn Glu
65                  70                  75                  80

Trp Leu Val Arg Gln Asp Ile Val Val Gly Asp Asn Val Ala Glu Ala
                85                  90                  95

Asn Glu Lys Ala Leu Asp Leu Leu Asn Lys Gly Val Asp Ser Leu Gly
            100                 105                 110

Phe Tyr Leu Lys Lys Val His Ile Asn Val Asp Thr Leu Ala Ala Leu
        115                 120                 125

Leu Lys Asp Ile Glu Leu Thr Ala Val Glu Leu Asn Phe Asn Cys Cys
    130                 135                 140

Ile Thr Arg Ala Ala Asp Leu Leu Ser Ala Phe Ser Ala Tyr Val Lys
145                 150                 155                 160

Lys Val Gly Ala Asp Pro Asn Lys Cys His Gly Ser Val Ser Tyr Asp
                165                 170                 175

Pro Phe Lys Lys Gln Leu Val Arg Gly Val Ser Asn Pro Asp Trp Val
            180                 185                 190

Lys Met Thr Leu Pro Val Met Asp Ala Ala Arg Glu Leu Pro Ala Phe
        195                 200                 205

Arg Val Leu Asn Val Asn Ala Val Asn Leu Ser Asp Ala Gly Ala Phe
    210                 215                 220

Ile Thr Gln Glu Leu Gly Tyr Ala Leu Ala Trp Gly Ala Glu Leu Leu
225                 230                 235                 240

Asp Lys Leu Thr Asp Ala Gly Tyr Lys Pro Glu Glu Ile Ala Ser Arg
                245                 250                 255

Ile Lys Phe Asn Phe Gly Ile Gly Ser Asn Tyr Phe Met Glu Ile Ala
            260                 265                 270

Lys Phe Arg Ala Ala Arg Trp Leu Trp Ala Gln Ile Val Gly Ser Tyr
        275                 280                 285

Gly Asp Gln Tyr Lys Asn Glu Thr Ala Lys Ile His Gln His Ala Thr
    290                 295                 300

Thr Ser Met Trp Asn Lys Thr Val Phe Asp Ala His Val Asn Leu Leu
305                 310                 315                 320

Arg Thr Gln Thr Glu Thr Met Ser Ala Ala Ile Ala Gly Val Asp Ser
                325                 330                 335

Ile Thr Val Leu Pro Phe Asp Val Thr Tyr Gln Gln Ser Asp Asp Phe
            340                 345                 350

Ser Glu Arg Ile Ala Arg Asn Gln Gln Leu Leu Leu Lys Glu Glu Cys
        355                 360                 365

His Phe Asp Lys Val Ile Asp Pro Ser Ala Gly Ser Tyr Tyr Ile Glu
    370                 375                 380

Thr Leu Thr Asn Ser Ile Gly Glu Glu Ala Trp Lys Leu Phe Leu Ser
385                 390                 395                 400

Val Glu Asp Ala Gly Gly Phe Thr Gln Ala Ala Glu Thr Ala Ser Ile
                405                 410                 415

Gln Lys Ala Val Asn Ala Ser Asn Ile Lys Arg His Gln Ser Val Ala
            420                 425                 430

Thr Arg Arg Glu Ile Phe Leu Gly Thr Asn Gln Phe Pro Asn Phe Thr
        435                 440                 445

Glu Val Ala Gly Asp Lys Ile Thr Leu Ala Gln Gly Glu His Asp Cys
    450                 455                 460
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Asn Cys Val Lys Ser Ile Glu Pro Leu Asn Phe Ser Arg Gly Ala Ser
465                 470                 475                 480

Glu Phe Glu Ala Leu Arg Leu Ala Thr Glu Lys Ser Gly Lys Thr Pro
                485                 490                 495

Val Val Phe Met Leu Thr Ile Gly Asn Leu Ala Met Arg Leu Ala Arg
            500                 505                 510

Ser Gln Phe Ser Ser Asn Phe Phe Gly Cys Ala Gly Tyr Lys Leu Ile
        515                 520                 525

Asp Asn Leu Gly Phe Lys Ser Val Glu Glu Gly Val Asp Ala Ala Leu
    530                 535                 540

Ala Ala Lys Ala Asp Ile Val Val Leu Cys Ser Ser Asp Asp Glu Tyr
545                 550                 555                 560

Ala Glu Tyr Ala Pro Ala Ala Phe Asp Tyr Leu Ala Gly Arg Ala Glu
                565                 570                 575

Phe Val Val Ala Gly Ala Pro Ala Cys Met Ala Asp Leu Glu Ala Lys
            580                 585                 590

Gly Ile Arg Asn Tyr Val His Val Lys Ser Asn Val Leu Glu Thr Leu
        595                 600                 605

Arg Ala Phe Asn Asp Lys Phe Gly Ile Arg
    610                 615


<210> SEQ ID NO 70
<211> LENGTH: 715
<212> TYPE: PRT
<213> ORGANISM: Porphyromonas gingivalis
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: NCBI / NP_905777
<309> DATABASE ENTRY DATE: 2010-06-29
<313> RELEVANT RESIDUES IN SEQ ID NO: (1)..(715)

<400> SEQUENCE: 70

Met Lys Pro Asn Tyr Lys Asp Ile Asp Ile Lys Ser Ala Gly Phe Val
1               5                   10                  15

Ala Lys Asp Ala Thr Arg Trp Ala Glu Glu Lys Gly Ile Val Ala Asp
            20                  25                  30

Trp Arg Thr Pro Glu Gln Ile Met Val Lys Pro Leu Tyr Thr Lys Asp
        35                  40                  45

Asp Leu Glu Gly Met Glu His Leu Asp Tyr Val Ser Gly Leu Pro Pro
    50                  55                  60

Phe Leu Arg Gly Pro Tyr Ser Gly Met Tyr Pro Met Arg Pro Trp Thr
65                  70                  75                  80

Ile Arg Gln Tyr Ala Gly Phe Ser Thr Ala Glu Glu Ser Asn Ala Phe
                85                  90                  95

Tyr Arg Arg Asn Leu Ala Ser Gly Gln Lys Gly Leu Ser Val Ala Phe
            100                 105                 110

Asp Leu Ala Thr His Arg Gly Tyr Asp Ala Asp His Ser Arg Val Val
        115                 120                 125

Gly Asp Val Gly Lys Ala Gly Val Ser Ile Cys Ser Leu Glu Asp Met
    130                 135                 140

Lys Val Leu Phe Asp Gly Ile Pro Leu Ser Lys Met Ser Val Ser Met
145                 150                 155                 160

Thr Met Asn Gly Ala Val Leu Pro Ile Leu Ala Phe Tyr Ile Asn Ala
                165                 170                 175

Gly Leu Glu Gln Gly Ala Lys Leu Glu Glu Met Ala Gly Thr Ile Gln
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            180                 185                 190

Asn Asp Ile Leu Lys Glu Phe Met Val Arg Asn Thr Tyr Ile Tyr Pro
        195                 200                 205

Pro Glu Phe Ser Met Arg Ile Ile Ala Asp Ile Phe Glu Tyr Thr Ser
    210                 215                 220

Gln Asn Met Pro Lys Phe Asn Ser Ile Ser Ile Ser Gly Tyr His Met
225                 230                 235                 240

Gln Glu Ala Gly Ala Thr Ala Asp Ile Glu Met Ala Tyr Thr Leu Ala
                245                 250                 255

Asp Gly Met Gln Tyr Leu Lys Ala Gly Ile Asp Ala Gly Ile Asp Val
            260                 265                 270

Asp Ala Phe Ala Pro Arg Leu Ser Phe Phe Trp Ala Ile Gly Val Asn
        275                 280                 285

His Phe Met Glu Ile Ala Lys Met Arg Ala Ala Arg Leu Leu Trp Ala
    290                 295                 300

Lys Ile Val Lys Ser Phe Gly Ala Lys Asn Pro Lys Ser Leu Ala Leu
305                 310                 315                 320

Arg Thr His Ser Gln Thr Ser Gly Trp Ser Leu Thr Glu Gln Asp Pro
                325                 330                 335

Phe Asn Asn Val Gly Arg Thr Cys Ile Glu Ala Met Ala Ala Ala Leu
            340                 345                 350

Gly His Thr Gln Ser Leu His Thr Asn Ala Leu Asp Glu Ala Ile Ala
        355                 360                 365

Leu Pro Thr Asp Phe Ser Ala Arg Ile Ala Arg Asn Thr Gln Ile Tyr
    370                 375                 380

Ile Gln Glu Glu Thr Leu Val Cys Lys Glu Ile Asp Pro Trp Gly Gly
385                 390                 395                 400

Ser Tyr Tyr Val Glu Ser Leu Thr Asn Glu Leu Val His Lys Ala Trp
                405                 410                 415

Thr Leu Ile Lys Glu Val Gln Glu Met Gly Gly Met Ala Lys Ala Ile
            420                 425                 430

Glu Thr Gly Leu Pro Lys Leu Arg Ile Glu Glu Ala Ala Ala Arg Thr
        435                 440                 445

Gln Ala Arg Ile Asp Ser His Gln Gln Val Ile Val Gly Val Asn Lys
    450                 455                 460

Tyr Arg Leu Pro Lys Glu Asp Pro Ile Asp Ile Leu Glu Ile Asp Asn
465                 470                 475                 480

Thr Ala Val Arg Lys Gln Gln Ile Glu Arg Leu Asn Asp Leu Arg Ser
                485                 490                 495

His Arg Asp Glu Lys Ala Val Gln Glu Ala Leu Glu Ala Ile Thr Lys
            500                 505                 510

Cys Val Glu Thr Lys Glu Gly Asn Leu Leu Asp Leu Ala Val Lys Ala
        515                 520                 525

Ala Gly Leu Arg Ala Ser Leu Gly Glu Ile Ser Asp Ala Cys Glu Lys
    530                 535                 540

Val Val Gly Arg Tyr Lys Ala Val Ile Arg Thr Ile Ser Gly Val Tyr
545                 550                 555                 560

Ser Ser Glu Ser Gly Glu Asp Lys Asp Phe Ala His Ala Lys Glu Leu
                565                 570                 575

Ala Glu Lys Phe Ala Lys Lys Glu Gly Arg Gln Pro Arg Ile Met Ile
            580                 585                 590
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Ala Lys Met Gly Gln Asp Gly His Asp Arg Gly Ala Lys Val Val Ala
        595                 600                 605

Thr Gly Tyr Ala Asp Cys Gly Phe Asp Val Asp Met Gly Pro Leu Phe
    610                 615                 620

Gln Thr Pro Glu Glu Ala Ala Arg Gln Ala Val Glu Asn Asp Val His
625                 630                 635                 640

Val Met Gly Val Ser Ser Leu Ala Ala Gly His Lys Thr Leu Ile Pro
                645                 650                 655

Gln Val Ile Ala Glu Leu Glu Lys Leu Gly Arg Pro Asp Ile Leu Val
            660                 665                 670

Thr Ala Gly Gly Val Ile Pro Ala Gln Asp Tyr Asp Phe Leu Tyr Gln
        675                 680                 685

Ala Gly Val Ala Ala Ile Phe Gly Pro Gly Thr Pro Val Ala Tyr Ser
    690                 695                 700

Ala Ala Lys Val Leu Glu Ile Leu Leu Glu Glu
705                 710                 715
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What is claimed is:

1. A method for producing branched-chain fatty acid comprising a methyl on one or more even number carbons, the method comprising culturing a cell comprising

(aa) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a polypeptide that catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA and/or (bb) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a polypeptide that catalyzes the conversion of succinyl-CoA to methylmalonyl-CoA,

under conditions allowing expression of the polynucleotide(s) and production of branched-chain fatty acid, wherein the cell produces more branched-chain fatty acid comprising a methyl on one or more even number carbons than an otherwise similar cell that does not comprise the polynucleotide(s).

2. The method of claim **1** further comprising extracting from culture the branched-chain fatty acid or a product of the branched-chain fatty acid.

3. The method of claim **2**, wherein the polypeptide that catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA is a propionyl-CoA carboxylase and/or the polypeptide that catalyzes the conversion of succinyl-CoA to methylmalonyl-CoA is a methylmalonyl-CoA mutase.

4. The method of claim **3**, wherein (i) the propionyl-CoA carboxylase is *Streptomyces coelicolor* PccB and AccA1 or PccB and AccA2 and/or (ii) the methylmalonyl-CoA mutase is *Janibacter* sp. HTCC2649 methylmalonyl-CoA mutase, *S. cinnamonensis* MutA and MutB, or *E. coli* Sbm.

5. The method of claim **3**, wherein (i) the methylmalonyl-CoA mutase comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence set forth in SEQ ID NOs: 3, 4, or 28 and/or (ii) the propionyl-CoA carboxylase comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence set forth in SEQ ID NOs: 9 and 10.

6. The method of claim **3**, wherein the cell comprises an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA mutase and further comprises an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA epimerase.

7. The method of claim **2**, wherein the cell further comprises an exogenous or overexpressed polynucleotide encoding an acyl transferase lacking polyketide synthesis activity and/or an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a thioesterase.

8. The method of claim **7**, wherein the acyl transferase is FabD, an acyl transferase domain of a polyketide synthase, or an acyl transferase domain of *Mycobacterium* mycocerosic acid synthase.

9. The method of claim **2**, wherein the cell has been modified to attenuate endogenous methylmalonyl-CoA mutase activity, endogenous methylmalonyl-CoA decarboxylase activity, and/or endogenous acyl transferase activity.

10. The method of claim **2**, wherein the cell produces a Type II fatty acid synthase.

11. The method of claim **10**, wherein the cell is *Escherichia coli*.

12. A branched-chain fatty acid produced by the method of claim **1**.

13. A cell comprising:

(i) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding an acyl transferase lacking polyketide synthesis activity, and

(ii) an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a propionyl-CoA carboxylase and/or an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA mutase,

wherein the polynucleotide(s) are expressed and the cell produces more branched-chain fatty acid comprising a methyl on one or more even number carbons than an otherwise similar cell that does not comprise the polynucleotide(s).

14. The cell of claim **13**, wherein (i) the propionyl-CoA carboxylase is *Streptomyces coelicolor* PccB and AccA1 or PccB and AccA2 and/or (ii) the methylmalonyl-CoA mutase

is *Janibacter* sp. HTCC2649 methylmalonyl-CoA mutase, *S. cinnamonensis* MutA and MutB, or *E. coli* Sbm.

15. The cell of claim **13**, wherein (i) the methylmalonyl-CoA mutase comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence set forth in SEQ ID NOs: 3, 4, or 28 and/or (ii) the propionyl-CoA carboxylase comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence set forth in SEQ ID NOs: 9 and 10.

16. The cell of claim **13**, wherein the cell comprises an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA mutase and further comprises an exogenous or overexpressed polynucleotide comprising a nucleic acid sequence encoding a methylmalonyl-CoA epimerase.

17. The cell of claim **13**, wherein the acyl transferase is FabD, an acyl transferase domain of a polyketide synthase, or an acyl transferase domain of *Mycobacterium* mycocerosic acid synthase.

18. The cell of claim **13**, wherein the cell further comprises an exogenous or overexpressed polynucleotide comprises a nucleic acid sequence encoding a thioesterase.

19. The cell of claim **13**, wherein the cell has been modified to attenuate endogenous methylmalonyl-CoA mutase activity, endogenous methylmalonyl-CoA decarboxylase activity, and/or endogenous acyl transferase activity.

20. The cell of claim **13**, wherein the cell is *Escherichia coli*.

* * * * *