(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 102666866 A
(43)申请公布日 2012.09.12

(21)申请号 201080052242.5

(22)申请日 2010.09.29

(30)优先权数据
61/246844 2009.09.29 US

(85)PCT申请进入国家阶段日
2012.05.17

(86)PCT申请的申请数据
PCT/US2010/050724 2010.09.29

(87)PCT申请的公布数据
WO2011/041415 EN 2011.04.07

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(51)Int.Cl.
C12P 7/16(2006.01)
C12P 7/42(2006.01)
C12N 9/04(2006.01)
C12N 9/88(2006.01)
C12N 15/52(2006.01)
C12N 1/16(2006.01)
C12N 1/21(2006.01)

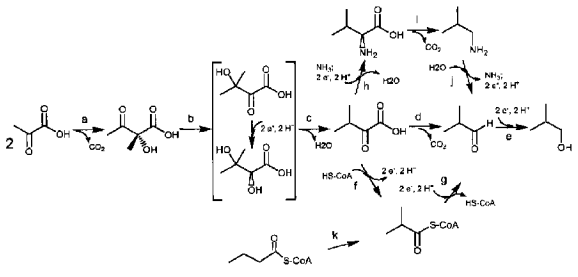
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(54)发明名称

使用高效酮醇酸还原异构酶发酵生产异丁醇

(57)摘要

已鉴定了酮醇酸还原异构酶,其提供了细菌和酵母体内高效异丁醇生物合成途径的步骤。这些KARI是通过分子系统发育分析鉴定的clade成员,称为SLSL Clade。



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1. 酵母细胞，包含至少一个核酸分子，所述核酸分子编码具有酮醇酸还原异构酶活性的多肽，其中所述多肽是 KARI 的 SLSL Clade 的成员。

2. 权利要求 1 的酵母细胞，其中所述 SLSL Clade 由细菌内源性的酮醇酸还原异构酶组成，所述细菌选自葡萄球菌属、李斯特菌属、肠球菌属、巨型球菌属、链球菌属、乳球菌属、明串珠菌属、乳杆菌属。

3. 权利要求 1 的酵母细胞，其中所述具有酮醇酸还原异构酶活性的多肽具有的氨基酸序列与选自 SEQ ID NO :2、4、6、8、10、12、14、16、18、20、22、24、26、28、30、32、34、36、38、40、42、44、46、48、50、52、54、56、58、60、62、64、66、68 和 245 的序列具有至少约 80％的同一性。

4. 权利要求 1 的酵母细胞，其中所述细胞是选自以下酵母属的成员 :糖酵母属、裂殖糖酵母属、汉逊酵母属、假丝酵母属、克鲁维酵母属、耶氏酵母属、伊萨酵母属和毕赤酵母属。

5. 生产异丁醇的微生物细胞，包含至少一个核酸分子，所述核酸分子编码具有酮醇酸还原异构酶活性的多肽，其中所述多肽是 KARI 的 SLSL Clade 的成员。

6. 权利要求 5 的微生物细胞，其中所述 SLSL Clade 由细菌内源性的酮醇酸还原异构酶组成，所述细菌选自葡萄球菌属、李斯特菌属、肠球菌属、巨型球菌属、链球菌属、乳球菌属、明串珠菌属、乳杆菌属。

7. 权利要求 5 的微生物细胞，其中所述编码酮醇酸还原异构酶活性的多肽具有的氨基酸序列与选自 SEQ ID NO :2、4、6、8、10、12、14、16、18、20、22、24、26、28、30、32、34、36、38、40、42、44、46、48、50、52、54、56、58、60、62、64、66、68 和 245 的序列具有至少约 80％的同一性。

8. 权利要求 5 的微生物宿主细胞，其中所述细胞是细菌细胞或酵母细胞。

9. 权利要求 8 的微生物宿主细胞，其中所述宿主细胞是选自以下属的细菌细胞 :埃希氏菌属、红球菌属、假单胞菌属、芽孢杆菌属、肠球菌属、乳球菌属、乳杆菌属、明串珠菌属、酒球菌属、小球菌属、链球菌属、梭菌属、发酵单胞菌属、沙门氏菌属、小球菌属、产碱菌属、克雷伯氏菌属、类芽胞杆菌属、节杆菌属、棒状杆菌属和短杆菌属。

10. 权利要求 8 的微生物宿主细胞，其中所述宿主细胞是选自以下属的酵母细胞 :糖酵母属、裂殖糖酵母属、汉逊酵母属、假丝酵母属、克鲁维酵母属、耶氏酵母属、伊萨酵母属和毕赤酵母属。

11. 用于将乙酰乳酸转化为二羟基异戊酸的方法，包括 :

a) 提供了包含至少一个核酸分子的酵母细胞，所述核酸分子编码具有酮醇酸还原异构酶活性的多肽，其中所述多肽是 KARI 的 SLSL Clade 的成员 ;并且

b) 使 (a) 中的酵母细胞与乙酰乳酸接触，其中产生 2,3- 二羟基异戊酸。

12. 权利要求 11 的方法，其中所述 SLSL Clade 由细菌内源性的酮醇酸还原异构酶组成，所述细菌选自葡萄球菌属、李斯特菌属、肠球菌属、巨型球菌属、链球菌属、乳球菌属、明串珠菌属、乳杆菌属。

13. 用于生产异丁醇的方法，包括 :

a) 提供了包含异丁醇生物合成途径的微生物细胞，所述微生物细胞包含至少一个核酸分子，所述核酸分子编码具有酮醇酸还原异构酶活性的多肽，其中所述多肽是 KARI 的 SLSL Clade 的成员 ;

b) 使步骤 (a) 中的微生物细胞在产生异丁醇的条件下生长。

14. 权利要求 13 的方法，其中所述 SLSL Clade 由细菌内源性的酮醇酸还原异构酶组成，所述细菌选自葡萄球菌属、李斯特菌属、肠球菌属、巨型球菌属、链球菌属、乳球菌属、明串珠菌属、乳杆菌属。

15. 权利要求 13 的方法，其中所述具有酮醇酸还原异构酶活性的多肽具有的氨基酸序列与选自 SEQ ID NO：2、4、6、8、10、12、14、16、18、20、22、24、26、28、30、32、34、36、38、40、42、44、46、48、50、52、54、56、58、60、62、64、66、68 和 245 的序列具有至少约 80％的同一性。

16. 酵母细胞，所述酵母细胞经工程化以具有至少一个灭活的丙酮酸脱羧酶基因并包含质粒，所述质粒具有的编码区与选自 SEQ ID NO：198、203、204、208 或 211 的质粒的编码区具有至少约 80％的同一性。

17. 酵母细胞，所述酵母细胞经工程化以具有至少一个灭活的丙酮酸脱羧酶基因并包含质粒，所述质粒具有的嵌合基因与选自 SEQ ID NO：198、203、204、208 或 211 的质粒的嵌合基因具有至少约 80％的同一性。

18. 质粒，所述质粒具有 SEQ ID NO：198、203、204、208 或 211 的序列。

使用高效酮醇酸还原异构酶发酵生产异丁醇

[0001] 相关申请的交叉引用

[0002] 本专利申请要求 2009 年 9 月 29 日提交的美国临时专利申请 61/246,844 的优先权，将所述文献全文以引用方式并入本文。

发明领域

[0003] 本发明涉及工业微生物学和发酵领域，具体地讲涉及异丁醇的生产。更具体地讲，发现酮醇酸还原异构酶 (KARI) 对乳酸菌和酵母中用于生产异丁醇的工程化异丁醇生物合成途径高度有效。

[0004] 发明背景

[0005] 丁醇是重要的工业化学品，可用作燃料添加剂、塑料工业中的化学原料以及食品和香料工业中的食品级萃取剂。每年，通过石油化学手段生产 100 至 120 亿磅的丁醇，并且对该日用化学品的需求可能还会增加。

[0006] 已经通过表达丁醇生物合成途径对微生物进行工程化以制备丁醇。异丁醇的生物合成途径在美国专利公布 US 20070092957 中公开。为了获得商业可用的异丁醇生产方法，需要非常高效的异丁醇途径。该途径的第二步通过酮醇酸还原异构酶 (KARI) 催化，所述酶将乙酰乳酸转化成二羟基异戊酸。具有高活性的 KARI 酶以及这些酶在异丁醇生物合成途径中的使用已经在美国专利公布 US20080261230A1 中公开。

[0007] 仍需要改善微生物中将乙酰乳酸转化成二羟基异戊酸中的步骤以最大化异丁醇的生产，所述微生物经工程化具有异丁醇生物合成途径。

[0008] 发明概述

[0009] 本发明提供表达酮醇酸还原异构酶 (KARI) 的微生物宿主细胞，其体内的乙酰乳酸高效转化成二羟基异戊酸，使得在具有工程化异丁醇生物合成途径的宿主细胞中生产较多的异丁醇。高效的 KARI 属于分子系统发育组，其包括乳酸乳球菌 (Lactococcus lactis) 和变异链球菌 (Streptococcus mutans)KARI。

[0010] 在本发明的一个方面，提供了包含至少一个核酸分子的酵母细胞，所述核酸分子编码具有酮醇酸还原异构酶活性的多肽，其中所述多肽是 KARI 的 SLSL Clade 的成员。在另一方面，酵母细胞是选自糖酵母属 (Saccharomyces)、裂殖糖酵母属 (Schizosaccharomyces)、汉逊酵母属 (Hansenula)、假丝酵母属 (Candida)、克鲁维酵母属 (Kluyveromyces)、耶氏酵母属 (Yarrowia) 和毕赤酵母属 (Pichia) 的酵母属的成员。

[0011] 在另一方面，所述 SLSL Clade 由细菌内源性的酮醇酸还原异构酶组成，所述细菌选自葡萄球菌属 (Staphylococcus)、李斯特菌属 (Listeria)、肠球菌属 (Enterococcus)、巨型球菌属 (Macrococcus)、链球菌属 (Streptococcus)、乳球菌属 (Lactococcus)、明串珠菌属 (Leuconostoc)、乳杆菌属 (Lactobacillus)。

[0012] 在另一方面，所述多肽具有酮醇酸还原异构酶活性，其氨基酸序列与选自 SEQ ID NO :2、4、6、8、10、12、14、16、18、20、22、24、26、28、30、32、34、36、38、40、42、44、46、48、50、52、54、56、58、60、62、64、66、68 和 245 的序列具有至少约 80％的同一性。

[0013] 本发明的另一方面是异丁醇生产微生物细胞，其包含至少一个核酸分子，所述核酸分子编码具有酮醇酸还原异构酶活性的多肽，其中所述多肽是 KARI 的 SLSL Clade 的成员。

[0014] 在另一方面，所述微生物细胞是选自以下属的细菌细胞：埃希氏菌属 (Escherichia)、红球菌属 (Rhodococcus)、假单胞菌属 (Pseudomonas)、芽孢杆菌属 (Bacillus)、肠球菌属 (Enterococcus)、乳球菌属 (Lactococcus)、乳杆菌属 (Lactobacillus)、明串珠菌属 (Leuconostoc)、酒球菌属 (Oenococcus)、小球菌属 (Pediococcus)、链球菌属 (Streptococcus)、梭菌属 (Clostridium)、发酵单胞菌属 (Zymomonas)、沙门氏菌属 (Salmonella)、小球菌属 (Pediococcus)、产碱菌属 (Alcaligenes)、克雷伯氏菌属 (Klebsiella)、类芽胞杆菌属 (Paenibacillus)、节杆菌属 (Arthrobacter)、棒状杆菌属 (Corynebacterium) 和短杆菌属 (Brevibacterium)。

[0015] 在另一方面，提供了将乙酰乳酸转化成二羟基异戊酸的方法，包括：

[0016] a) 提供了包含至少一个核酸分子的酵母细胞，所述核酸分子编码具有酮醇酸还原异构酶活性的多肽，其中所述多肽是 KARI 的 SLSL Clade 的成员；并且

[0017] b) 使所述 (a) 的酵母细胞与乙酰乳酸接触，其中产生 2,3- 二羟基异戊酸。

[0018] 在另一方面，提供了生产异丁醇的方法，包括：

[0019] a) 提供了包含异丁醇生物合成途径的微生物细胞，其包含至少一个核酸分子，所述核酸分子编码具有酮醇酸还原异构酶活性的多肽，其中所述多肽是 KARI 的 SLSL Clade 的成员；

[0020] b) 使步骤 (a) 的微生物细胞在使异丁醇产生的条件下生长。

[0021] 本文也提供了经工程化以具有至少一个灭活丙酮酸脱羧酶基因并包含质粒的酵母细胞，所述质粒具有选自 SEQ ID NO：198、203、204、208 或 211 的质粒编码区，以及具有与选自 SEQ ID NO：198、203、204、208 或 211 的质粒编码区至少约 80％，至少约 90％，至少约 95％或至少约 99％的同一性的那些质粒编码区。也提供了经工程化以具有至少一个灭活丙酮酸脱羧酶基因并包含质粒的酵母细胞，所述质粒具有选自 SEQ ID NO：198、203、204、208 或 211 的质粒嵌合基因，以及具有与选自 SEQ ID NO：198、203、204、208 或 211 的质粒嵌合基因至少约 80％，至少约 85％，至少约 90％，至少约 95％或至少约 99％的同一性的那些嵌合基因。也提供了具有 SEQ ID NO：198、203、204、208 或 211 的序列的质粒以及包含与上述序列具有至少约 80％，至少约 90％，至少约 95％或至少约 99％的同一性的那些序列的质粒。

[0022] 附图简述

[0023] 从下文的发明详述、附图和附带的序列描述中可较充分地理解本发明的多个实施方案，它们形成本专利申请的一部分。

[0024] 图 1 示出了三个不同的异丁醇生物合成途径。

[0025] 图 2 示出了 KARI 的分子系统发育树，其包括 667 个具有 95％序列同一性临界值的序列。圆圈外的符号标志着 SLSL Clade。

[0026] 图 3 示出了图 2 的一部分系统发育树，其包括 KARI 序列的 SLSL Clade。菱形标志着乳杆菌目 (Lactobacillales) 的成员，圆圈标志着芽孢杆菌目 (Bacillales) 的成员。在图例中列出了与每个次分支具有 95％的同一性的菌种。

[0027] 图4示出了具有不同KARI酶的异丁醇生产酵母的生长曲线图。SYK：单酵母ILV5；SLK：单乳酸乳球菌(L. lactis)IlvC；SPK：单Pf-5 ilvC。

[0028] 图5示出了具有不同KARI酶的异丁醇生产酵母的异丁醇滴度。SYK：单酵母ILV5；SLK：单乳酸乳球菌(L. Lactis)IlvC；SPK：单Pf-5 ilvC。

[0029] 根据下面的发明详述和附带的序列描述可以更充分地理解本发明，下面的发明详述和所附的序列描述形成本专利申请的一部分。

[0030] 下列序列符合37 C.F.R. 1.821-1.825(“对含有核酸序列和/或氨基酸序列公开的专利申请的要求—序列规则”)并且符合世界知识产权组织(WIPO)标准ST.25(1998)和EPO和PCT的序列表要求(规则5.2和49.5(a-bis)，以及行政性指示的208节和附录C)。用于核苷酸和氨基酸序列数据的符号和格式遵循在37 C.F.R. §1.822中所列出的规定。

[0031] 表1：高效KARI的编码区和蛋白质的序列号

[0032]

说明	SEQ ID NO: 核酸	SEQ ID NO: 氨基酸
头葡萄球菌(*Staphylococcus capitis*) *SK14*	1	2
表皮葡萄球菌(*Staphylococcus epidermidis*) *M23864-W1*	3	4
人葡萄球菌(*Staphylococcus hominis*) *SK119*	244	245
金黄色葡萄球菌金黄亚种(*Staphylococcus aureus subsp. aureus*) TCH130	5	6
沃氏葡萄球菌(*Staphylococcus warneri*) *L37603*	7	8
表皮葡萄球菌(*Staphylococcus epidermidis*) *W23144*	9	10
腐生性葡萄球菌腐生亚种(*Staphylococcus saprophyticus subsp. Saprophyticus*) *ATCC15305*	11	12
肉葡萄球菌肉亚种(*Staphylococcus carnosus subsp. Carnosus*) *TM300*	13	14

[0033]

单核细胞增多性李斯特菌（*Listeria monocytogenes*）*EGD-e*	15	16
格氏李斯特菌（*Listeria grayi*）*DSM 20601*	17	18
铅黄肠球菌（*Enterococcus casseliflavus*）*EC30*	19	20
鹑鸡肠球菌（*Enterococcus gallinarum*）*EG2*	21	22
溶酪巨型球菌（*Macrococcus caseolyticus*）*JCSC5402*	23	24
前庭链球菌（*Streptococcus vestibularis*）	25	26
变异链球菌（*Streptococcus mutans*）*UA159*	27	28
格登链球菌（*Streptococcus gordonii str*），*cgakkus sybstr. CH1*	29	30
猪链球菌（*Streptococcus suis*）*89/1591*	31	32
婴儿链球菌婴儿亚种（*Streptococcus infantarius subsp. infantarius*）*ATCC BAA-102*	33	34
乳酸乳球菌乳脂亚种（*Lactococcus lactis subsp. cremoris*）*MG1363*	35	36
乳酸乳球菌（*Lactococcus lactis*）	37	38
肠膜明串珠菌肠膜亚种（*Leuconostoc mesenteroides subsp mesenteroides*）*ATCC8293*	39	40
布氏乳杆菌（*Lactobacillus buchneri*）*ATCC 11577*	41	42
溶血性葡萄球菌（*Staphylococcus haemolyticus*）*JCSC1435*	43	44
表皮葡萄球菌（*Staphylococcus epidermidis*）*ATCC12228*	45	46
肺炎链球菌（*Streptococcus pneumoniae*）*CGSP14*	47	48
肺炎链球菌（*Streptococcus pneumoniae*）*TIGR4*	49	50
血链球菌（*Streptococcus sanguinis*）*SK36*	51	52
唾液链球菌（*Streptococcus salivarius*）*SK126*	53	54
嗜热链球菌（*Streptococcus thermophilus*）*LMD-9*	55	56
肺炎链球菌（*Streptococcus pneumoniae*）*CCRI 1974M2*	57	58
乳酸乳球菌乳酸亚种（*Lactococcus lactis subsp. lactis*）*Il1403*	59	60
肠膜明串珠菌乳脂亚种（*Leuconostoc mesenteroides subsp cremoris*）*ATCC19254*	61	62
肠膜明串珠菌乳脂亚种（*Leuconostoc mesenteroides subsp cremoris*）	63	64
短乳杆菌格雷夫斯亚种（*Lactobacillus brevis subsp. gravesensis*）*ATCC27305*	65	66
乳酸乳球菌乳脂亚种（*Lactococcus lactis subsp lactis*）*NCDO2118*	67	68

[0034] 表 2 表达编码区和蛋白质的 SEQ ID NO

[0035]

说明	SEQ ID NO: 核酸	SEQ ID NO: 氨基酸
ALS，得自枯草芽孢杆菌（*Bacillus subtilis*）	69	70
ALS，得自经优化用于植物乳杆菌（Lactobacillus plantarum）的枯草芽孢杆菌（*Bacillus subtilis*）编码区	71	70*
ALS，得自肺炎克雷伯氏菌（*Klebsiella pneumoniae*）（*budB*）	72	73
ALS，得自乳酸乳球菌（*Lactococcus lactis*）	74	75
ALS，得自金黄色葡萄球菌（*Staphylococcus aureus*）	76	77
ALS，得自单核细胞增多性李斯特菌（*Listeria monocytogenes*）	78	79
ALS，得自变异链球菌（*Streptococcus mutans*）	80	81
ALS，得自嗜热链球菌（*Streptococcus thermophilus*）	82	83
ALS，得自纤细弧菌（*Vibrio angustum*）	84	85
ALS，得自蜡状芽孢杆菌（*Bacillus cereus*）	86	87
KARI，得自荧光假单胞菌（*Pseudomonas fluorescens*）*ilvC PF5*	88	89
KARI，得自荧光假单胞菌（*Pseudomonas fluorescens*）*ilvC PF5* 密码子，其经优化用于植物乳杆菌（*L. plantarum*）	90	89*
KARI，得自荧光假单胞菌（*Pseudomonas fluorescens*）*ilvC PF5* 密码子，其经优化用于啤酒糖酵母（*S. cerevisiae*）	91	89*
KARI，得自啤酒糖酵母（Saccharomyces cerevisiae）ILV3	92	93
DHAD，得自乳酸乳球菌（*Lactococcus lactis*）*ilvD*	94	95
DHAD，得自变异链球菌（*Streptococcus mutans*）*ilvD*	96	97
DHAD，得自啤酒糖酵母（*Saccharomyces cerevisiae*）ILV3	98	99
支链酮酸脱羧酶，得自乳酸乳球菌（*Lactococcus lactis*）*kivD*	100	101
乳酸乳球菌（*Lactococcus lactis*）*kivD*，其经优化用于植物乳杆菌（*L. plantarum*）	102	101*
仲醇脱氢酶，得自木糖氧化无色杆菌（*Achromobacter xylosoxidans*）sadB	103	104
木糖氧化无色杆菌（*A. xylosoxidans*）sadB，其经优化用于植物乳杆菌（*L. plantarum*）	105	104*
马肝醇脱氢酶 *ADH*，其经密码子优化用于啤酒糖酵母（*S. cerevisiae*）	106	107
Tn5 转座酶	108	109

[0036] * 由天然序列或最优化序列编码的相同蛋白质序列

[0037] SEQ ID NO:110 是质粒 pFP996 的序列。

[0038] SEQ ID NO:111-121、123-126、130、131、133、134、136-141、143-148、151-154、156-159、161-163、165、166、168、170-173、177-181、186-197、199-202、205、206、209、210、213-222、224-243 是 PCR 和测序引物。

[0039] SEQ ID NO:122 是 pyrF 序列。

[0040] SEQ ID NO:127 是核糖体结合位点 (RBS)。

[0041] SEQ ID NO:128 是质粒 pDM20-ilvD（乳酸乳球菌 (L. lactis)）的序列。

[0042] SEQ ID NO:129 质粒 pDM1 的序列。

[0043] SEQ ID NO:132 是 PCR 片段的序列，该片段包括乳酸乳球菌 (Lactococcus lactis) 的 RBS 和 ilvD 编码区。

[0044] SEQ ID NO:135 是右同源臂 DNA 片段，其包含 suf 操纵子 (sufC 和部分 sufD) 的 5′ 部分。

[0045] SEQ ID NO:142 是左同源臂 DNA 片段，其包含天然 suf 启动子和 feoBA 操纵子中的上游序列。

[0046] SEQ ID NO:149 是质粒 pTN6 的序列。

[0047] SEQ ID NO:150 是 Tn5IE-loxP-cm-Pspac-loxP 盒的序列。

[0048] SEQ ID NO:155 是 Pnpr 启动子。

[0049] SEQ ID NO:160 是 Pnpr-tnp 融合 DNA 片段。

[0050] SEQ ID NO:164 是 PgroE 启动子序列。

[0051] SEQ ID NO:167 是 PCR 片段，其包含 kivD(o) 编码区以及 RBS。

[0052] SEQ ID NO:169 是 DNA 片段，其包含 RBS 和 sadB(o) 编码区。

[0053] SEQ ID NO:174 是质粒 pFP352 的序列。

[0054] SEQ ID NO:175 是质粒 pDM5 的序列。

[0055] SEQ ID NO:176 是 lacI-PgroE/lacO 片段。

[0056] SEQ ID NO:182 是质粒 pDM5-PldhL1-ilvC(乳酸乳球菌 (L.lactis)) 的序列。

[0057] SEQ ID NO:183 是 DNA 片段，其包括 PF5-ilvC 密码子的 RBS 和编码区，所述密码子经优化以用于植物乳杆菌 (L.plantarum) 表达。

[0058] SEQ ID NO:184 是质粒 pFP996-PldhL1 的序列。

[0059] SEQ ID NO:185 是 PldhL1-ilvC(荧光假单胞菌 (P.fluorescens)PF5)DNA 片段。

[0060] SEQ ID NO:198 是质粒 pYZ090 的序列。

[0061] SEQ ID NO:203 是质粒 pLH475-IlvC(乳酸乳球菌 (L.lactis)) 的序列。

[0062] SEQ ID NO:204 是质粒 pYZ091 的序列。

[0063] SEQ ID NO:207 是质粒 pLH532 的序列。

[0064] SEQ ID NO:208 是质粒 pYZ058 的序列。

[0065] SEQ ID NO:211 是质粒 pYZ067 的序列。

[0066] SEQ ID NO:212 是 pUC19-URA3r 载体的序列。

[0067] SEQ ID NO:223 是 ilvD-FBA1t 片段的序列。

[0068] 发明详述

[0069] 本发明涉及经工程化用于改善异丁醇生产的重组微生物宿主细胞。异丁醇是用于替代化石燃料的重要化合物。

[0070] 以下缩写和定义将用于说明书和权利要求的判读。

[0071] 如本文所用，术语“包含”、“含有”、“包括”、“涵盖”、“具有”、“具备”、“包容”或“容纳”或其任何其它变型旨在非排他性的包括。例如，包含一系列元素的组合物、混合物、工艺、方法、制品或设备不必仅限于那些元素，而可以包括其它未明确列出的元素，或此类组合物、混合物、工艺、方法、制品或设备固有的元素。此外，除非有相反的明确说明，“或”是指包含性的“或”，而不是指排他性的“或”。例如，以下任何一种情况均满足条件 A 或 B :A 是

真实的（或存在的）且B是虚假的（或不存在的），A是虚假的（或不存在的）且B是真实的（或存在的），以及A和B都是真实的（或存在的）。

[0072] 同样，涉及元素或组分实例（即次数）的数目在本发明元素或组分前的不定冠词“一个”或“一种”旨在是非限制性的。因此，应将“一个”或“一种”理解为包括一个或至少一个，并且元素或组分的词语单数形式也包括复数指代，除非有数字明显表示单数。

[0073] 如本文所用，术语“发明”或“本发明”是非限制性术语，并且不旨在意指本发明的任何单独实施方案，而是涵盖如本说明书和权利要求所述的所有可能的实施方案。

[0074] 如本文所用，用术语“约”修饰本发明的成分或反应物的数量时是指数值量的变化，它们可能发生在，例如，典型的测量和用于制备浓缩液或实际使用溶液的液体处理程序中；这些程序中的偶然误差中；制造、来源、或用于制备组合物或实施方法的成分的纯度的差异中；等。术语“约”还包括由于对于起因于特定起始混合物的组合物的不同平衡条件而不同的量。无论是否通过术语“约”来修饰，权利要求包括量的等同量。在一个实施方案中，术语“约”指在报告数值10％范围内，优选地在报告数值5％范围内。

[0075] 术语“异丁醇生物合成途径”是指从丙酮酸产生异丁醇的酶途径。

[0076] 术语“SLSL Clade”指通过分子系统发育分析鉴定为KARI序列的分支，所述序列包括得自葡萄球菌属(Staphylococcus)、李斯特菌属(Listeria)、链球菌属(Streptococcus)、乳球菌属(Lactococcus)、明串珠菌属(Leuconostoc)、肠球菌属(Enterococcus)、巨型球菌属(Macrococcus)和乳杆菌属(Lactobacillus)的KARI。图1和图2分别示出SLSL Clade与其它KARI以及SLSL Clade自身的关系。

[0077] 术语“碳底物”或“可发酵碳底物”指能够被本发明的宿主生物体代谢的碳源，并且特别是选自以下的碳源：单糖、低聚糖和多糖。

[0078] 术语“基因”指能够被表达为特定蛋白质的核酸片段，其任选包括编码序列前的调节序列(5′非编码序列)和编码序列后的调节序列(3′非编码序列)。“天然基因”指自然状态下与其自身的调控序列一起的基因。“嵌合基因”指为非天然基因的任何基因，包含天然状态下不一起存在的调控序列和编码序列。因此，嵌合基因可包含源自不同来源的调控序列和编码序列，或源自相同来源、但排列方式与天然存在的排列方式不同的调控序列和编码序列。“内源性基因”指在生物体基因组中处于其天然位置的天然基因。“外来基因”或“异源基因”指正常情况下不存在于宿主生物中，但是通过基因转移导入宿主生物或经某些方法修饰而改变其天然状态如改变其表达的基因。外来基因可以包含插入到非天然生物体内的天然基因或嵌合基因。“转基因”是已通过转化方法导入基因组内的基因。

[0079] 如本文所用，术语“编码区”是指编码特定氨基酸序列的DNA序列。“合适的调节序列”指位于编码序列的上游(5′非编码序列)、中间或下游(3′非编码序列)的核苷酸序列，其可影响相关编码序列的转录、RNA加工或稳定性、或翻译。调节序列可以包括启动子、翻译前导序列、内含子、多腺苷酸化识别序列、RNA加工位点、效应子结合位点和茎-环结构。

[0080] 术语“启动子”指能够控制编码序列或功能性RNA表达的DNA序列。一般来讲，编码序列位于启动子序列的3′端。启动子可整体源于天然基因，或由源于天然存在的不同启动子的不同元件构成，或甚至可包含合成的DNA片段。本领域内的技术人员应当理解，不同的启动子可以在不同的组织或细胞类型中，或在不同的发育阶段，或响应不同的环境条件

或生理条件而引导基因的表达。引起基因在大部分时间内在大多数细胞类型中表达的启动子通常称为“组成型启动子”。还应当进一步认识到，由于在大多数情况下调控序列的确切边界尚未完全确定，因此不同长度的 DNA 片段可能具有相同的启动子活性。

[0081] 术语“可操作地连接”指单个核酸片段上的核酸序列的关联，以便其中一个核酸序列的功能受到另一个核酸序列的影响。例如，当启动子能够影响编码序列的表达（即，该编码序列受到该启动子的转录控制）时，则该启动子与该编码序列可操作地连接。编码序列可以按有义或反义的取向可操作地连接至调控序列。

[0082] 如本文所用，术语“表达”指转录和有义 RNA(mRNA) 的稳定积聚。表达也可指将 mRNA 翻译成多肽。

[0083] 如本文所用，术语“转化”指将核酸分子转化到宿主细胞中，可将其以质粒形式或整合进基因组中保持。含有转化核酸分子的宿主细胞被称为“转基因”或“重组”或“转化”细胞。

[0084] 如本文所用，术语“质粒”和“载体”是指通常携带有不属于细胞中心代谢的部分的基因的染色体外元件，并且通常是环状双链 DNA 分子的形式。这类元件可为源自任何来源的自主复制序列、基因组整合序列、噬菌体或其它单链或双链 DNA 或 RNA 的核苷酸序列（可为线性或环状），其中多个核苷酸序列已连接或重组进入独特构建体中，该独特构建体能够将所选基因产物的启动子片段和 DNA 序列与相应的 3′ 末端非翻译序列一起导入细胞中。

[0085] 如本文所用，术语“密码子简并性”指允许核苷酸序列在不影响所编码的多肽的氨基酸序列的情况下发生变化的遗传密码的性质。技术人员非常了解具体宿主细胞在使用核苷酸密码子来确定给定氨基酸时所表现出的“密码子偏倚性”。因此，当合成编码区用以改善在宿主细胞中的表达时，希望对编码区进行设计，使得其密码子使用频率接近该宿主细胞优选的密码子使用频率。

[0086] 术语“密码子优化的”在其涉及用于转化不同宿主的核酸分子的编码区时是指在不改变由 DNA 编码的多肽的情况下，改变核酸分子的编码区中的密码子以反映宿主生物体通常的密码子使用。

[0087] 如本文所用，“分离的核酸片段”或“分离的核酸分子”将可以互换使用，并且将指单链或双链的 RNA 或 DNA 聚合体，任选地含有合成的、非天然的或改变的核苷酸碱基。DNA 聚合物形式的分离的核酸片段可由 cDNA、基因组 DNA 或合成 DNA 的一个或多个片段构成。

[0088] 当在合适的温度和溶液离子强度条件下单链形式的核酸片段可以退火至另一核酸片段时，核酸片段“可杂交”至另一核酸片段，例如 cDNA、基因组 DNA 或 RNA 分子。杂交条件和洗涤条件是众所周知的并例示于 Sambrook，J，Fritsch，E.F. 和 Maniatis，T. Molecular Cloning：A Laboratory Manual，第 2 版，Cold Spring Harbor Laboratory：Cold Spring Harbor，NY(1989)，尤其是其中的第 11 章和表 11.1（全文以引用方式并入本文）。温度和离子强度条件决定了杂交的“严格度”。可以调节严格条件以筛选中度相似的片段（例如来自远缘生物的同源序列），到筛选高度相似的片段（例如从近缘生物复制功能性酶的基因）。杂交后的洗涤可确定严格条件。一组优选的条件使用一系列洗涤步骤，开始是 6X SSC，0.5% SDS 在室温洗涤 15 分钟，然后用 2X SSC，0.5% SDS 在 45℃重复 30 分钟，然后用 0.2X SSC，0.5% SDS 在 50℃重复洗涤两次，每次 30 分钟。一组较优选的严格条件使用较高温度，其中洗涤步骤与上述那些步骤相同，不同的是最后两次用 0.2X SSC，0.5% SDS 洗涤 30 分

钟的洗涤温度提高到60℃。另一组优选的高严格条件是最后两次洗涤是在65℃下用0.1X SSC,0.1% SDS进行。例如,另一组严格条件包括在0.1X SSC,0.1% SDS中于65℃下杂交并用2X SSC,0.1% SDS洗涤,随后用0.1X SSC,0.1% SDS洗涤。

[0089] 杂交需要两种核酸含有互补序列,但是取决于杂交的严格度,碱基之间可能会发生错配。用于使核酸杂交的合适严格性取决于核酸的长度和互补的程度,所述长度和互补程度是本领域内所熟知的变量。两条核苷酸序列之间的相似性或同源性程度越高,具有那些序列的核酸的杂交体的Tm值就越大。核酸杂交的相对稳定性(对应较高的Tm)按以下顺序依次降低:RNA:RNA、DNA:RNA、DNA:DNA。就长度大于100个核苷酸的杂交体而言,已经获得了计算Tm的方程式(参见Sambrook等人,同上,9.50-9.51)。对于较短核酸(即寡核苷酸)的杂交,错配的位置变得更重要,并且寡核苷酸的长度决定了其特异性(参见Sambrook等人,同上,11.7-11.8)。在一个实施方案中,可杂交核酸的长度为至少约10个核苷酸。可杂交核酸的最小长度优选地为至少约15个核苷酸;更优选地为至少约20个核苷酸;并且最优选地长度为至少约30个核苷酸。此外,技术人员将认识到,必要时可根据诸如探针长度之类的因素来调节温度和洗涤溶液的盐浓度。

[0090] 氨基酸或核苷酸序列的“基本部分”指这样的部分,该部分包括的多肽的氨基酸序列或基因的核苷酸序列足以通过推定而鉴定所述多肽或基因,所述鉴定或者可以由本领域技术人员通过人工评价序列来完成,或者可以利用诸如BLAST(Altschul, S.F.,等人,J.Mol.Biol.,215:403-410(1993))的算法通过计算机自动化的序列比对和鉴定进行。一般来讲,为了推测鉴定多肽或核酸序列是否与已知的蛋白质或基因同源,需要有十个或更多邻接氨基酸或者三十个或更多核苷酸的序列。此外,对于核苷酸序列,包含20-30个邻接核苷酸的基因特异性寡核苷酸探针可用于序列依赖性的基因鉴定(如DNA杂交)和基因分离(如细菌菌落或噬斑的原位杂交)的方法中。此外,约17个碱基的短寡核苷酸可在PCR中用作扩增引物,以便获得包含该引物的特定核酸片段。因此,核苷酸序列的“基本部分”所包含的序列应足以特异性地鉴定和/或分离包含该序列的核酸片段。本说明书教导了编码特定蛋白质的完整氨基酸和核苷酸序列。受益于本文所报道的序列,技术人员现在可使用全部公布序列或它们的基本部分用于本领域技术人员已知的目的。因此,本发明包括在附随序列表中报道的完全序列,以及那些上述序列的主要部分。

[0091] 术语“互补的”用于描述核苷酸碱基之间能够彼此杂交的关系。例如,对于DNA,腺嘌呤与胸腺嘧啶互补,并且胞嘧啶与鸟嘌呤互补。

[0092] 如本领域所熟知的,术语“%同一性”是两条或更多条多肽序列之间或两条或更多条多核苷酸序列之间的关系,该关系通过对序列进行比较确定。在本领域中,“同一性”还表示多肽或多核苷酸序列之间序列关联的程度,根据具体情况,它由这些序列的序列串之间的匹配程度确定。“同一性”和“相似性”可容易地通过已知方法计算出来,所述的方法包括但不限于以下文献中所描述的那些:1.)Computational Molecular Biology(Lesk, A.M. 编辑)Oxford University:NY(1988);2.)Biocomputing:Informatics and Genome Projects(Smith, D.W. 编辑)Academic:NY(1993);3.)Computer Analysis of Sequence Data, Part I(Griffin, A.M. 和 Griffin, H.G. 编辑)Humania:NJ(1994);4.)Sequence Analysis in Molecular Biology(von Heinje, G. 编辑)Academic(1987);和5.)Sequence Analysis Primer(Gribskov, M. 和 Devereux, J. 编辑)Stockton:NY(1991)。

[0093] 设定确定同一性的优选方法来用于给出待测试序列之间的最佳匹配。确定同一性和相似性的方法在可公开获得的计算机程序中编成了代码。序列比对和%同一性计算可以用LASERGENE生物信息学计算软件包(LASERGENE bioinformatics computing suite(DNASTAR Inc.,Madison,WI))中的MegAlign™程序进行。序列的多重比对使用“Clustal比对方法”进行,该方法涵盖若干个不同的算法,包括对应于称为Clustal V比对方法的“Clustal V比对方法”,该方法描述于Higgins和Sharp,(CABIOS.5:151-153(1989);Higgins,D.G.等人,Comput.Appl.Biosci.,8:189-191(1992))中,并且可以在LASERGENE生物信息学计算软件包(DNASTAR Inc.)中的MegAlign™程序中找到的比对方法。对于多重比对,默认值对应于空位罚分(GAP PENALTY)=10和缺口长度罚分(GAP LENGTH PENALTY)=10。用Clustal方法进行逐对比对和蛋白质序列的%同一性计算的默认参数为KTUPLE=1、空位罚分=3、窗口(WINDOW)=5和DIAGONALS SAVED=5。对于核酸,这些参数为KTUPLE=2、空位罚分=5、窗口=4和DIAGONALS SAVED=4。在用Clustal V程序进行序列比对后,有可能通过观察相同程序中的“序列距离”表来获得“%同一性”。此外,也可以使用“Clustal W比对方法”,该方法相当于称为Clustal W(在Higgins和Sharp,CABIOS;5:151-153(1989);Higgins,D.G.等人,Comput.Appl.Biosci.8:189-191(1992)中有所描述)和可以在LASERGENE生物信息学计算软件包(DNASTAR Inc.)中的MegAlign™ v6.1程序中找到的比对方法。用于多重比对的默认参数(空位罚分=10、缺口长度罚分=0.2、延迟发散序列(Delay Divergen Seqs)(%)=30、DNA转换权重(DNA Transition Weight)=0.5、蛋白质权重矩阵(Protein Weight Matrix)=Gonnet系列和DNA权重矩阵(DNA Weight Matrix)=IUB)。在使用Clustal W程序对序列进行比对之后,可通过查看同一程序中的“序列距离”表来获得“%同一性”。

[0094] 本领域的技术人员非常清楚,多种程度的序列同一性可用于例如从其它物种中鉴定多肽,其中这类多肽具有相同或相似的功能或活性。同一性百分比的可用的实例包括但不限于:24%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、或95%、或24%至100%之间的任何整数百分比,例如25%、26%、27%、28%、29%、30%、31%、32%、33%、34%、35%、36%、37%、38%、39%、40%、41%、42%、43%、44%、45%、46%、47%、48%、49%、50%、51%、52%、53%、54%、55%、56%、57%、58%、59%、60%、61%、62%、63%、64%、65%、66%、67%、68%、69%、70%、71%、72%、73%、74%、75%、76%、77%、78%、79%、80%、81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%,它们可用于描述本发明。合适的核酸片段不但具有上述同源性,而且通常编码具有至少50个氨基酸,优选至少100个氨基酸,更优选至少150个氨基酸,更优选至少200个氨基酸,并且最优选至少250个氨基酸的多肽。

[0095] 术语“序列分析软件”指可用于分析核苷酸或氨基酸序列的任何计算机算法或软件程序。“序列分析软件”可商购获得或独立开发。典型的序列分析软件将包括但不限于:1)GCG程序软件包(Wisconsin Package Version 9.0,Genetics Computer Group(GCG),Madison,WI);2)BLASTP,BLASTN,BLASTX(Altschul等人,J.Mol.Biol.,215:403-410(1990));3.)DNASTAR(DNASTAR,Inc.Madison,WI);4.)Sequencher(Gene Codes Corporation,Ann Arbor,MI);和5.)整合了Smith-Waterman算法的FASTA程序(W.R.Pearson,Comput.Methods Genome Res.,[Proc.Int.Symp.](1994),召开年份:1992,

111-20。编辑 :Suhai,Sandor.Plenum :New York,NY)。在本专利申请案的上下文中应当理解,使用序列分析软件进行分析时,除非另外指明,否则分析结果将基于所参考程序的“默认值”。在此所用的“默认值”将指在首次初始化软件时软件最初加载的任何值或参数集。

[0096] 本文使用的标准重组 DNA 和分子克隆技术是本领域熟知的并且已经在如下文献中描述 :Sambrook,J.,Fritsch,E.F. 和 Maniatis,T.,Molecular Cloning :A Laboratory Manual,第二版,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,NY(1989)(hereinafter“Maniatis”); 并 且 Silhavy,T.J.,Bennan,M.L. 和 Enquist,L.W.,Experiments with Gene Fusions,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,NY(1984) ;以及 Ausubel,F.M. 等人,Current Protocols in Molecular Biology,Greene Publishing Assoc. 和 Wiley-Interscience 出版 (1987)。所用的更多方法见于 Methods in Enzymology,第 194 卷,Guide to Yeast Genetics 和 Molecular and Cell Biology(Part A,2004,Christine Guthrie 和 Gerald R.Fink(编辑),Elsevier Academic Press,San Diego,CA)。

[0097] 具有高体内效率的 KARI 的发现

[0098] 生产异丁醇的生物合成途径在美国专利公布 US 20070092957 中公开,如图 1 所示。期望最大化生物合成途径的步骤以最大化异丁醇生产。图 1 中所有途径的第二个步骤是通过酮醇酸还原异构酶 (KARI) 将乙酰乳酸转化成二羟基异戊酸。申请人已经鉴定了 KARI,当其在酵母和细菌的异丁醇生物合成途径中使用时,提供比以前使用其它 KARI 获得的异丁醇产量更高的产量。

[0099] 在表达乳酸乳球菌 (Lactococcus lactis)KARI(SEQ ID NO :67 的编码序列 ;SEQ ID NO :68 的蛋白质) 作为表达异丁醇途径的 KARI 的酵母中,发现异丁醇的产量大于使用荧光假单胞菌 (Pseudomonas fluorescens)KARI(SEQ ID NO :91 的编码序列 ;SEQ ID NO :89 的蛋白质) 或啤酒糖酵母 (Saccharomyces cerevisiae)KARI(SEQ ID NO :92 的编码序列 ;SEQ ID NO :93 的蛋白质) 的异丁醇产量。在测试条件下的异丁醇产量为至少约两倍。与此相反,乳酸乳球菌 (L.lactis)KARI 的体外活性小于荧光假单胞菌 (荧光假单胞菌 (P.fluorescens))KARI 的体外活性。在表达来自乳酸乳球菌 (Lactococcus lactis)(SEQ ID NO :67 的编码序列 ;SEQ ID NO :68 的蛋白质)、变异链球菌 (Streptococcus mutans)(SEQ ID NO :27 的编码序列 ;SEQ ID NO :28 的蛋白质)、嗜热链球菌 (Streptococcus thermophilis)(SEQ ID NO :55 的编码序列 ;SEQ ID NO :56 的蛋白质) 或肠膜明串珠菌 (Leuconostoc mesenteroides)(SEQ ID NO :39 的编码序列 ;SEQ ID NO :40 的蛋白质) 的 KARI 作为表达异丁醇途径的 KARI 的植物乳杆菌 (Lactobacillus plantarum) 中,发现异丁醇的产量大于使用荧光假单胞菌 (Pseudomonas fluorescens)KARI(SEQ ID NO :90 的编码序列 ;SEQ ID NO :89 的蛋白质) 的异丁醇产量。在测试条件下的异丁醇产量提高了至少三倍。异丁醇产量的提高量可取决于多个因素如宿主菌株、存在的其它异丁醇途径酶、培养基和培养条件而不同。与使用荧光假单胞菌 (Pseudomonas fluorescens)KARI 相比,当使用高效 KARI 时在细菌和酵母中的异丁醇产量提高了约两倍。异丁醇产量可提高 2 倍、3 倍、4 倍或更多倍。

[0100] 可用于本发明细胞和方法的、高效用于异丁醇生产的 KARI 是那些通过 KARI 氨基酸序列的分子系统发育分析鉴定的一组成员。对 KARI 序列进行分子系统发育分析,

所述序列通过荧光假单胞菌(Pseudomonas fluorescens)KARI Pf-5(SEQ ID NO:89)的BLAST分析从公开数据库中收集得到。使用公开可用的Jalview程序(Waterhouse等人(2009)Bioinformatics doi:10.1093/bioinformatics/btp033)的邻接法从KARI序列中生成多序列比对(MSA)并从MSA中生成序列的系统发育树。图2示出了所得系统发育树,其中具有95%或更高的同一性的KARI序列用单序列表示。通过这个KARI序列分析,发现如上所述测试的来自乳酸乳球菌(Lactococcus lactis)(SEQ ID NO:68)、变异链球菌(Streptococcus mutans)(SEQ ID NO:28)、嗜热链球菌(Streptococcus thermophilis)(SEQ ID NO:56)和肠膜明串珠菌(Leuconostoc mesenteroides)(SEQ ID NO:40)的KARI都是明确定义的KARI系统发育分枝、或clade,所述KARI此外还包括来自其它菌株乳球菌(Lactococcus)、链球菌(Streptococcus)和明串珠菌(Leuconostoc)的KARI,以及来自葡萄球菌(Staphylococcus)、李斯特菌(Listeria)、肠球菌(Enterococcus)、巨型球菌(Macrococcus)和乳杆菌(Lactobacillus)菌种的KARI。因此将这个KARI的clade鉴定为SLSL Clade并在图1中示出。此外,图3示出了包含SLSL Clade的部分系统发育树。

[0101] 在本发明的细胞和方法中可使用SLSL Clade成员的任何KARI。本文鉴定的这个系统发育分支的成员包括来自不同菌种葡萄球菌(Staphylococcus)、李斯特菌(Listeria)、链球菌(Streptococcus)、乳球菌(Lactococcus)、明串珠菌(Leuconostoc)、肠球菌(Enterococcus)、巨型球菌(Macrococcus)和乳杆菌(Lactobacillus)的KARI,包括在表1中列出为SEQ ID NO:2、4、6、8、10、12、14、16、18、20、22、24、26、28、30、32、34、36、38、40、42、44、46、48、50、52、54、56、58、60、62、64、66、68和245的那些KARI。这些KARI的编码区序列分别具有SEQ ID NO:1、3、5、7、9、11、13、15、17、19、21、23、25、27、29、31、33、35、37、39、41、43、45、47、49、51、53、55、57、59、61、63、65、67和244。与表1中的任何序列具有99%或更高的同一性的大多数序列未被列出,但是它们也可用于本发明的细胞和方法,并且易于由本领域的技术人员利用如上所述的生物信息学分析来鉴定。因此,与表1中的序列具有至少约99%的同一性的序列可用于本发明的细胞。

[0102] 附加的KARI属于KARI的SLSL CLade,它们在文献和生物信息学数据库中可容易地被鉴定,这是技术人员熟知的。使用生物信息学方法鉴定编码和/或蛋白质序列通常通过BLAST(如上所述)搜索包含KARI编码序列或编码氨基酸序列的公开可用的数据库进行,例如本文提供的那些。如上所述的分子系统发育分析可用确定是否KARI是SLSL Clade的成员。附加的KARI包括是SLSL Clade成员的那些,它们具有与SEQ ID NO:2、4、6、8、10、12、14、16、18、20、22、24、26、28、30、32、34、36、38、40、42、44、46、48、50、52、54、56、58、60、62、64、66、68和245中的任何KARI氨基酸序列至少约80-85%、85%-90%、90%-95%、或至少约96%、97%、98%、或99%的氨基酸序列同一性。同一性基于Clustal W比对方法,所述方法采用空位罚分=10、空位长度罚分=0.1的默认参数和Gonnet 250系列的蛋白质权重矩阵。

[0103] 此外,本文所述的序列或本领域所述的那些序列还可用于鉴定天然的其它同源物。例如每个编码本文所述的核酸片段的KARI可用于分离编码同源蛋白的基因。使用序列依赖性规程分离同源基因是本领域熟知的。序列依赖性规程的实例包括但不限于:1)核酸杂交方法;2)DNA和RNA扩增方法,例如多种核酸扩增技术[例如,聚合酶链反应(PCR),Mullis等人,美国专利4,683,202;连接酶链反应(LCR),Tabor,S.等人,Proc.Acad.

Sci.USA 82:1074(1985);或链置换扩增反应(SDA),Walker 等人,Proc.Natl.Acad.Sci.U.S.A.,89:392(1992)];和 3) 文库构建和互补筛选方法。

[0104] 酵母和细菌细胞中的 KARI 表达

[0105] 上述的任何 KARI 可在酵母或细菌细胞中表达以将乙酰乳酸转化成二羟基异戊酸,提供异丁醇生物合成途径中的步骤。可为宿主细胞的酵母细胞包括但不限于属于以下的属:糖酵母属(Saccharomyces)、裂殖糖酵母属(Schizosaccharomyces)、汉逊酵母属(Hansenula)、假丝酵母属(Candida)、克鲁维酵母属(Kluyveromyces)、耶氏酵母属(Yarrowia)、伊萨酵母属(Issatchenkia)和毕赤酵母属(Pichia)。可为宿主细胞的细菌细胞包括但不限于属于以下的属:埃希氏菌属(Escherichia)、红球菌属(Rhodococcus)、假单胞菌属(Pseudomonas)、芽孢杆菌属(Bacillus)、肠球菌属(Enterococcus)、乳球菌属(Lactococcus)、乳酸菌属(Lactobacillus)、明串珠菌属(Leuconostoc)、酒球菌属(Oenococcus)、小球菌属(Pediococcus)、链球菌属(Streptococcus)、梭菌属(Clostridium)、发酵单胞菌属(Zymomonas)、沙门氏菌属(Salmonella)、小球菌属(Pediococcus)、产碱菌属(Alcaligenes)、克雷伯氏菌属(Klebsiella)、类芽胞杆菌属(Paenibacillus)、节杆菌属(Arthrobacter)、棒状杆菌属(Corynebacterium)和短杆菌属(Brevibacterium)。尤其有用的宿主细胞是乳酸菌(LAB)如乳球菌(Lactococcus)、乳酸菌(Lactobacillus)、明串珠菌(Leuconostoc)、酒球菌(Oenococcus)、小球菌(Pediococcus)和链球菌(Streptococcus)。

[0106] 上述表达 KARI 编码区的方法是本领域技术人员熟知的。用于酵母中基因表达的方法是已知的并描述于例如Methods in Enzymology,第194卷,Guide to Yeast Genetics and Molecular and Cell Biology(Part A,2004,Christine Guthrie和Gerald R Fink(编辑),Elsevier Academic Press,San Diego,CA)。

[0107] 通常通过可操作地将编码区连接到用于在靶细胞中表达的启动子上,期望的 KARI 的编码区被构造为嵌合基因的一部分。嵌合表达基因可包括终止控制区,并且可包括用于细菌细胞表达的核糖体结合位点。编码区可经密码子优化以在本领域技术人员已知的工程化特定宿主细胞中表达。

[0108] 可用于在酵母中表达的启动子为例如组成型启动子 FBA1、TDH3、ADH1 和 GPM1,以及诱导型启动子 GAL1、GAL10 和 CUP1。可用于在酵母中表达的嵌合基因构建体的适用转录终止子包括但不限于 FBA1t、TDH3t、GPM1t、ERG10t、GAL1t、CYC1t 和 ADH1t。

[0109] 可将合适的启动子、转录终止子和编码区克隆进大肠杆菌-酵母穿梭载体中并转化到酵母细胞中。这些载体既可大肠杆菌菌株中增殖也可在酵母菌株中增殖。载体通常包含选择性标记和在目标宿主中允许自主复制或染色体整合的序列。在酵母中通常使用的质粒是穿梭载体 pRS423、pRS424、pRS425 和 pRS426(美国典型培养物保藏中心,Rockville,MD),它们包含大肠杆菌复制起点(例如,pMB1)、酵母 2μ 复制起点,以及用于营养选择的标记。这四种载体的选择标记是His3(载体pRS423)、Trp1(载体pRS424)、Leu2(载体pRS425)和Ura3(载体pRS426)。携带嵌合基因的表达载体的构建,既可以通过大肠杆菌中标准的分子克隆技术进行,也可以通过酵母中的缺口修复重组方法进行。嵌合基因可从质粒中表达或被整合进细胞基因组中。

[0110] 用于在细菌细胞中表达嵌合基因的启动子有很多并且对本领域的技术人员来说

是熟悉的，它们包括但不限于：lac、ara、tet、trp、lPL、lPR、T7、tac 和 trc 启动子（对于在大肠杆菌、产碱菌属和假单胞菌属中的表达是有用的）；amy、apr 和 npr 启动子，以及多种对于在枯草芽孢杆菌、地衣芽孢杆菌和浸麻类芽孢杆菌中的表达有用的噬菌体启动子；nisA（对于在革兰氏阳性菌中的表达是有用的，Eichenbaum 等人 Appl. Environ. Microbiol. 64(8)：2763-2769(1998)）；以及合成的 P11 启动子（对于在植物乳杆菌中的表达是有用的，Rud 等人，Microbiology 152：1011-1019(2006)）。此外，植物乳杆菌的 ldhL1 和 fabZ1 启动子可用于在 LAB 中表达嵌合基因。fabZ1 启动子引导操纵子的转录，该操纵子具有第一基因，fabZ1，其编码 (3R)- 羟基肉豆蔻酰 -[酰基载体蛋白] 脱水酶。

[0111] 终止控制区也可源于优选宿主的天然的多个细菌基因。

[0112] 用于转化多种细菌细胞的载体是常见的并且可从公司商购获得，例如 EPICENTRE ® (Madison, WI)，Invitrogen Corp. (Carlsbad, CA)、Stratagene(La Jolla, CA) 和 New England Biolabs, Inc. (Beverly, MA)。某些载体能够在多种宿主细菌中复制并且能通过缀合进行转移。pRK404 的完整的并且带注释的序列和三种相关载体：pRK437、pRK442 和 pRK442(H) 是可用的。这些衍生物已被证明是在革兰氏阴性菌中进行遗传操纵的有用工具 (Scott 等人，Plasmid 50(1)：74-79(2003))。广宿主范围的 IncP4 质粒 RSF1010 的几种衍生质粒也可获得，其具有在一系列革兰氏阴性菌中发挥功能的启动子。质粒 pAYC36 和 pAYC37 具有连同多个克隆位点一起的活性启动子，使得在革兰氏阴性菌中的异源基因能够表达。对于枯草芽孢杆菌和乳杆菌的转化有用的一些载体包括：pAMβ1 及其衍生物 (Renault 等人，Gene 183：175-182(1996)；和 O'Sullivan 等人，Gene 137：227-231(1993))；pMBB1 和 pHW800，pMBB1 的衍生物 (Wyckoff 等人 Appl. Environ. Microbiol. 62：1481-1486(1996))；pMG1，接合质粒 (Tanimoto 等人，J. Bacteriol. 184：5800-5804(2002))；pNZ9520(Kleerebezem 等人，Appl. Environ. Microbiol. 63：4581-4584(1997))；pAM401(Fujimoto 等人，Appl. Environ. Microbiol. 67：1262-1267(2001))；和 pAT392(Arthur 等人，Antimicrob. Agents Chemother. 38：1899-1903(1994))。几种来源于植物乳杆菌 (Lactobacillus plantarum) 的质粒也已被报道 (vanKranenburg 等人，Appl. Environ. Microbiol. 71(3)：1223-1230(2005))。

[0113] 可利用本领域已知的方法如电穿孔和缀合将载体或质粒导入宿主细胞中。

[0114] 用于异丁醇生产的宿主细胞

[0115] 经工程化具有异丁醇生物合成途径的包括本文所述的 KARI 的酵母和细菌细胞可具有附加的修改形式。可制备改善宿主细胞的任何修改形式，例如提高向异丁醇合成的溶剂的修改形式。

[0116] 例如，可利用的酵母细胞可经工程化以具有至少一个灭活的丙酮酸脱羧酶 (PDC) 基因以生成 pdc- 细胞，以便减少丙酮酸向乙醛的转化并使较多的丙酮酸能转向异丁醇途径。酵母可具有一个或多个编码丙酮酸脱羧酶的基因。例如在光滑假丝酵母 (Candida glabrata) 和粟酒裂殖酵母 (Schizosaccharomyces pombe) 中有一个编码丙酮酸脱羧酶的基因，然而有三个丙酮酸脱羧酶的同功酶，它们由糖酵母属 (Saccharomyces) 中的 PDC1、PCD5 和 PDC6 基因编码。如果使用的酵母细胞具有多于一种的表达（活性）PDC 基因，那么灭活每个活性 PDC 基因，从而制备 pdc- 细胞。例如在啤酒糖酵母 (S. cerevisiae) 中可灭活 PDC1、PDC5 和 PDC6 基因。假如在使用的发酵条件下 PDC 基因是无活性的，例如 PDC6，那

么这个基因不需要进行灭活。

[0117] 糖酵母 (Saccharomyces) 菌株无丙酮酸脱羧酶活性，它们可得自 ATCC，具有 Accession #200027 和 #200028。此外，如美国专利申请公布 20090305363 和本文实施例 1 所述，酵母可经工程化以灭活内源活性 PDC 基因，上述专利申请以引用方式并入本文。

[0118] 此外，可使用的酵母细胞可经工程化以具有降低的葡萄糖阻遏。在克拉布特里阳性酵母中发生的葡萄糖阻遏是在高水平葡萄糖的存在下，涉及呼吸代谢的基因表达和非葡萄糖碳源的利用发生阻遏的现象 (Gancedo(1998)Microbiol.Mol.Bio.Rev.62 :334-361)。在提交于 2009 年 9 月 29 日的美国临时专利申请 61/246709 中公开了以下发现 :具有降低的葡萄糖阻遏以及 pdc- 表型和异丁醇途径的酵母细胞具有改善的异丁醇产量并且在高水平葡萄糖中的生长改善。如以引用方式并入本文的美国临时专利申请 61/246709 所述，可通过以下方法降低葡萄糖阻遏，例如 :(1) 改变涉及葡萄糖阻遏效应的转录因素的表达。例如，提高 Hap1 转录激活因子的表达或降低 Mig1 或 Mig2 转录阻遏蛋白的表达 ;(2) 降低 GRR1(抗葡萄糖阻遏蛋白) 的表达，它是 SCF 泛素连接酶络合物的组分并且似乎是葡萄糖阻遏途径的主要因素，(3) 通过灭活己糖转运蛋白基因降低葡萄糖转运能力，所述基因包括 HXT1、HXT2、HX3、HXT4、HXT5、HXT6 和 / 或 HXT7，或 (4) 删除己糖激酶 2 基因 (HXK2)。改变上文提到的基因的表达可通过本领域熟知的方法进行工程化，包括如本文实施例 1 所例示的方法。

[0119] 例如，可使用的乳酸菌 (LAB) 细胞可经工程化以具有降低的乳酸脱氢酶活性，以便减少从丙酮酸生产的乳酸以促进丙酮酸转向如美国专利申请公布 20100112655 所述的另一个途径，该专利申请以引用方式并入本文。LAB 可具有一个或多个基因，通常具有一个、两个或三个基因，所述基因编码乳酸脱氢酶。例如，植物乳杆菌 (Lactobacillus plantarum) 具有三个编码乳酸脱氢酶的基因，它们称为 ldhL2、ldhD 和 ldhL1。乳酸乳球菌 (Lactococcus lactis) 具有一个编码乳酸脱氢酶的基因，其称为 ldhL，并且戊糖片球菌 (Pediococcus pentosaceus) 具有两个称为 ldhD 和 ldhL 的基因。当多于一种的乳酸脱氢酶基因在使用的生长条件下具有活性时，这些活性基因中的每一个可被修改以减少表达，如本文实施例 1 所述。

[0120] 此外，可工程化 LAB 宿主细胞以提高铁硫簇形成蛋白的表达以改善异丁醇途径的需铁硫簇二羟酸脱水酶的活性，如美国专利申请 20100081182 所公开的那样，该专利申请以引用方式并入本文。例如，可如本文实施例 2 所述提高编码铁硫簇形成蛋白的内源 suf 操纵子的表达。

[0121] 可用于本文提供的细胞的附加修改形式包括如美国专利申请公布 20090305363(以引用方式并入本文) 所述的降低甘油 3- 磷酸脱氢酶活性的修改形式，通过恩特纳 - 道德洛夫途径提供提高的碳流量的宿主细胞修改形式或如美国专利申请公布 20100120105(以引用方式并入本文) 所述降低等效平衡。具有涉及酵母线粒体中支链氨基酸生物合成的某些活性降低酶的酵母细胞在美国专利申请公布 20100129887(以引用方式并入本文) 中进行了描述，并且具有活性提高的异源蛋白 (其需要结合铁硫簇作为它们的活性辅因子) 的酵母菌株在美国专利申请公布 20100081179(以引用方式并入本文) 中进行了描述。其它修改形式包括在编码具有双功能己糖激酶活性的多肽的内源多核苷酸中的修改形式，如美国临时申请 61/290,639 所述，整合至少一个多核苷酸的修改形式，所述多

核苷酸编码催化利用丙酮酸的生物合成途径中的步骤的多肽，如美国临时申请 61/380563 所述（两个提到的临时申请全文以引用方式并入本文）。

[0122] 此外，在编码影响铁硫簇生物合成的多肽的内源性基因中包含至少一个缺失、突变、和 / 或取代的宿主细胞在美国临时专利申请 61/305333（以引用方式并入本文）中进行了描述，并且包含编码具有磷酸酮醇酶活性的多肽的异源多核苷酸的宿主细胞和包含编码具有磷酸转乙酰酶活性的多肽的异源多核苷酸的宿主细胞在美国临时专利申请 61/356379 中进行了描述。

[0123] 用于工程化具有上述修改形式的宿主细胞的方法是本领域熟知的。用于基因表达的方法包括上述那些用于表达 KARI 的方法。用于基因灭活的方法包括但不限于删除整个或部分编码基因，将 DNA 片段插入编码基因（插入启动子或编码区）使得编码蛋白不表达，将添加终止密码子或移码的突变导入编码区使得功能性蛋白不表达，以及将一个或多个突变导入编码区以改变氨基酸使得表达的蛋白无功能。此外，可通过表达反义 RNA 或干涉 RNA 阻止表达，并且可导入导致共抑制的构建体。

[0124] 异丁醇生产

[0125] 本发明细胞具有 KARI，如本文所述它在体内利用生物合成途径高效地制备异丁醇，所述生物合成途径如公开于美国专利申请公布 20070092957 A1 中的途径，其以引用方式并入本文并在图 1 中示出。

[0126] 如 US 20070092957 A1 中所述，在实施例中异丁醇生物合成途径的步骤包括下列转化：

[0127] - 丙酮酸至乙酰乳酸（图 1 途径步骤 a），其例如通过已知 EC 编号 2.2.1.69 的乙酰乳酸合酶 (ALS) 催化；

[0128] - 乙酰乳酸至 2,3- 二羟基异戊酸（图 1 途径步骤 b），其例如通过已知 EC 编号 1.1.1.86 的乙酰羟酸异构还原酶，也称为酮醇酸还原异构酶 (KARI) 催化；

[0129] -2,3- 二羟基异戊酸至 α - 酮异戊酸（图 1 途径步骤 c），其例如通过已知 EC 编号 4.2.1.9 的乙酰羟酸脱水酶，也称为二羟酸脱水酶 (DHAD) 催化；

[0130] - α - 酮异戊酸至异丁醛（图 1 途径步骤 d），其例如通过已知 EC 编号 4.1.1.72 或 4.1.1.1 的支链 α - 酮酸脱羧酶催化；以及

[0131] - 异丁醛至异丁醇（图 1 途径步骤 e），其例如通过已知 EC 编号 1.1.1.265 的支链醇脱氢酶催化，但是所述酶也可被归类为其它醇脱氢酶（具体地讲，EC 1.1.1.1 或 1.1.1.2）。

[0132] 就选择性途径的步骤 f、g、h、l、j 和 k 而言，将底物转化为产物以及这些反应中所涉及的酶描述于 US 20070092957 A1 中。

[0133] 可用于表达这些酶以及两个附加异丁醇途径的那些酶的基因在 US 20070092957 A1 中进行了描述，并且如上所述的可用的附加基因可在文献中以及使用生物信息学方法鉴定，这是技术人员熟知的。此外，如上所述，本文提供的序列可用于利用序列依赖性的规程分离编码同源蛋白的基因，这是本领域熟知的。

[0134] 例如，可使用的一些代表性的 ALS 酶，包括由芽孢杆菌属 (Bacillus) 的 alsS 和克雷伯氏菌属 (Klebsiella) 的 budB 编码的那些酶 (Gollop 等人，J.Bacteriol.172(6)：3444-3449(1990)；Holtzclaw 等人，J.Bacteriol.121(3)：917-922(1975))。本文提供了

得自枯草芽孢杆菌 (Bacillus subtilis)(DNA :SEQ ID NO :69 ;蛋白质 :SEQ ID NO :70)、肺炎克雷伯氏菌 (Klebsiella pneumoniae)(DNA :SEQ ID NO :72 ;蛋白质 :SEQ ID NO :73) 和乳酸乳球菌 (Lactococcus lactis)(DNA :SEQ ID NO :74 ;蛋白质 :SEQ ID NO :75) 的 ALS,以及枯草芽孢杆菌 (Bacillus subtilis)als 编码区,其经优化用于在植物乳杆菌 (Lactobacillus plantarum) 中表达 (SEQ ID NO :71)。可用的附加 als 编码区和编码蛋白包括得自金黄色葡萄球菌 (Staphylococcus aureus)(DNA :SEQ ID NO :76 ;蛋白质 :SEQ ID NO :77)、单核细胞增多性李斯特菌 (Listeria monocytogenes)(DNA :SEQ ID NO :78 ;蛋白质 :SEQ ID NO :79)、变异链球菌 (Streptococcus mutans)(DNA :SEQ ID NO :80 ;蛋白质 :SEQ ID NO :81)、嗜热链球菌 (Streptococcus thermophilus)(DNA :SEQ ID NO :82 ;蛋白质 :SEQ ID NO :83)、纤细弧菌 (Vibrio angustum)(DNA :SEQ ID NO :84 ;蛋白质 :SEQ ID NO :85) 和蜡状芽孢杆菌 (Bacillus cereus)(DNA :SEQ ID NO :86 ;蛋白质 :SEQ ID NO :87) 的那些。可使用编码乙酰乳酸合酶的任何 als 基因,所述酶与将丙酮酸转化成乙酰乳酸的那些序列 SEQ ID NO :70、73、75、77、79、81、83、85 或 87 中的任何一个具有至少约 80-85%、85% -90%、90% -95%、或至少约 96%、97% 或 98% 的序列同一性。同一性基于 Clustal W 比对方法,所述方法采用空位罚分 = 10、空位长度罚分 = 0.1 的默认参数和 Gonnet 250 系列的蛋白质权重矩阵。

[0135] 此外,以引用方式并入本文的美国专利申请公布 20090305363 提供了系统发育树描述的乙酰乳酸合酶,它们是枯草芽孢杆菌 (B.subtilis)AlsS 序列的 100 个最近邻居,可使用它们中的任何一个。在本发明菌株中可用的附加 Als 序列可在文献中或生物信息学数据库中鉴定,这是技术人员熟知的。使用生物信息学方法鉴定编码和 / 或蛋白质序列通常通过 BLAST(如上所述) 搜索包含已知的 Als 编码序列或编码氨基酸序列的公开可用的数据库进行,例如本文提供的那些。基于如上所述的 Clustal W 比对方法进行鉴定。此外,可利用本文列出的序列或本领域所述的那些序列鉴定如上所述的其它天然同源物。

[0136] 酵母中的乙酰乳酸合酶的细胞质内表达通过用包含编码乙酰乳酸合酶蛋白的序列的基因转化来完成,所述序列无线粒体靶向信号序列。用于酵母中基因表达的方法是本领域已知的 (参见例如 Methods in Enzymology,第 194 卷,Guide to Yeast Genetics and Molecular and Cell Biology Part A,2004, Christine Guthrie 和 Gerald R.Fink(编辑),Elsevier Academic Press,San Diego,CA)。使用嵌合基因 (包括启动子和终止子)、载体、克隆方法和整合方法的表达如上所述。

[0137] 上文描述了可使用的 KARI 酶。也预期附加的 KARI 酶可与本文所述的 KARI 酶一起使用。适用的 KARI 酶包括在美国专利申请公布 20080261230、20090163376 和 20100197519 中描述的那些酶,所有上述专利申请均以引用方式并入本文。

[0138] 例如,可用的 DHAD 酶可为得自乳酸乳球菌 (Lactococcus lactis)(DNA :SEQ ID NO :94 ;蛋白质 SEQ ID NO :95) 或变异链球菌 (Streptococcus mutans)(DNA :SEQ ID NO :96 ;蛋白质 SEQ ID NO :97) 的 ilvD 基因、或者得自啤酒糖酵母 (Saccharoomyces cerevisiae)(DNA :SEQ ID NO :98 ;蛋白质 SEQ ID NO :99) 的 ILV3 基因。可用于获取可用的附加 DHAD 序列的附加 DHAD 序列在以引用方式并入本文的美国专利申请公布 20100081154 中公开。此参考文献也包括对获取可用的附加 DHAD 序列的描述。

[0139] 例如,可用的支链酮酸脱羧酶包括得自乳酸乳球菌 (Lactococcus lactis)(DNA :

SEQ ID NO:100;蛋白质 SEQ ID NO:101)的 kivD 基因的酶、以及经密码子优化以在植物乳杆菌(Lactobacillus plantarum)(SEQ ID NO:102)中表达的乳酸乳球菌(L.lactis)kivD 编码区和如上所述可由本领域技术人员利用生物信息学方法鉴定的其它酶。

[0140] 例如,可用的支链醇脱氢酶是已知 EC 编号 1.1.1.265 的酶,但是所述酶也可被归类为其它醇脱氢酶(具体地讲,EC 1.1.1.1 或 1.1.1.2)。这些酶利用 NADH(还原型烟酰胺腺嘌呤二核苷酸)和/或 NADPH 作为电子供体,并且在 US20070092957 A1 中提供了可用的支链醇脱氢酶和它们的编码区的序列。

[0141] 此外,用于将异丁醛转化成异丁醇的最终步骤的酶是新丁醇脱氢酶 sadB,其分离自细菌的环境分离物,所述细菌经鉴定为木糖氧化无色杆菌(Achromobacter xylosoxidans)(DNA:SEQ ID NO:103,蛋白质 SEQ ID NO:104),其在以引用方式并入本文的美国专利申请公布 20090269823 中公开。可使用经优化以在植物乳杆菌(L.plantarum)(SEQ ID NO:105)中表达的 sadB 编码区。此外,得自马肝的醇脱氢酶(HADH;其经密码子优化以在啤酒糖酵母(S.cerevisiae)中表达;DNA:SEQ ID NO:106;蛋白质 SEQ ID NO:107)以及其它酶易于由本领域技术人员利用如上所述的生物信息学方法鉴定。附加的醇脱氢酶在以引用方式并入本文的美国临时专利申请 61/290,636 中描述。

[0142] 在基本上无乳酸脱氢酶活性的 LAB 细胞中活性改善的 DHAD 在以引用方式并入本文的美国专利申请公布 20100081183 中公开。此外,提高铁硫簇形成蛋白的表达以改善 DHAD 活性在以引用方式并入本文的美国专利申请公布 2010-0081182 中公开。

[0143] 对于利用所公开的生物合成途径的异丁醇生产,US 20070092957 A1 中描述了嵌合基因的构建以及通过植物乳杆菌(Lactobacillus plantarum)例示的 LAB 的基因工程改造。用于途径酶表达的嵌合基因可存在于细胞的复制质粒中或整合进细胞基因组中,这是本领域的技术人员熟知的。对于利用所公开的生物合成途径的异丁醇生产,US 20070092957 A1 中还描述了嵌合基因的构建和酵母,例如啤酒糖酵母的基因工程。基因构建和表达的进一步描述在上文和本文实施例中。

[0144] 用于生产的生长

[0145] 本文公开的细菌和酵母细胞可在发酵培养基中生长以生产异丁醇。为了最大化产量,用作生产宿主的菌株优选地具有增强的异丁醇耐受性并具有高碳水化合物利用速率。这些特征可通过诱变和选择、基因工程化赋予或可为天然存在的。

[0146] 细胞在包含合适碳底物的发酵培养基中生长。合适的底物可包括但不限于:单糖,例如葡萄糖和果糖;低聚糖,例如乳糖或蔗糖;多糖,例如淀粉、纤维素或它们的混合物;以及来自可再生原料的未纯化混合物,例如干酪乳清渗透物、玉米浆、糖用甜菜糖蜜及大麦麦芽。其它碳底物可包括乙醇、乳酸盐、琥珀酸盐或甘油。此外,可发酵的糖类可通过预处理和糖化过程来源于可再生的纤维质的或木质纤维质的生物质,例如,如美国专利申请公布 2007/0031918A1 中所述,该专利以引用方式并入本文。因此,预期本发明中所利用的碳源可涵盖各种含碳底物并将仅受限于生物体的选择。

[0147] 除了合适的碳源外,发酵培养基还必须含有本领域技术人员已知的适于培养物生长并促进生产异丁醇所必需的酶途径的矿物、盐、辅因子、缓冲剂和其它组分。

[0148] 培养条件

[0149] 通常细菌细胞在合适培养基中,在约 25℃至约 40℃的温度范围内生长,而酵母细

胞在合适培养基中，在约 20℃至约 37℃的温度范围内生长。适用的生长培养基是常见的商业制备培养基，并且用于培养特定细胞所用的合适培养基将是微生物或发酵科学领域的技术人员已知的

[0150] 就细菌而言，适于发酵的 pH 范围在 pH 5.0 到 pH 9.0 之间，其中 pH 6.0 至 pH 8.0 优选作为起始条件。就酵母而言，适于发酵的 pH 范围在 pH3.0 到 pH 7.5 之间，其中 pH 4.5 至 pH 6.5 优选作为起始条件。

[0151] 发酵可以在有氧或厌氧条件下进行，其中厌氧或微氧条件是优选的。

[0152] 预期可以或者采用分批发酵、补料分批发酵或者采用连续发酵工艺来实施异丁醇生产，并且任何已知的发酵模式均将适用。另外，预期可以将细胞固定在底物上而作为完整的细胞催化剂并让其经受发酵条件以生产异丁醇。

[0153] 用于从发酵培养基中分离异丁醇的方法

[0154] 生物生产的异丁醇可使用本领域中已知的方法用于 ABE 发酵（参见例如，Durre, Appl.Microbiol.Biotechnol.49 :639-648(1998)，Groot 等 人，Process.Biochem.27 : 61-75(1992)，以及本文参考）。例如，可通过离心、过滤、滗析等方法从发酵培养基中移除固体。然后，可使用诸如蒸馏、共沸蒸馏、液液萃取、吸附、汽提、膜蒸发、或全蒸发的方法从发酵培养基中分离异丁醇。

实施例

[0155] 本发明将在以下实施例中得到进一步阐述。应当理解，尽管这些实施例说明了本发明的优选实施方案，但仅是以例证的方式给出的。通过上述论述和这些实施例，本领域的技术人员可确定本发明的必要特征，并且在不脱离本发明的实质和范围的前提下，可对本发明进行各种变化和修改以使其适应多种用途和条件。

[0156] 缩写的含义如下：“s”是指秒，“min”是指分钟，“h”是指小时，“psi”是指磅每平方英寸，“nm”是指纳米，“d”是指天，“μL”是指微升，“mL”是指毫升，“L”是指升，“mm”是指毫米，“nm”是指纳米，“mM”是指毫摩尔每升，“M”是指摩尔每升，“mmol”是指毫摩尔，“μmol”是指微摩尔，“g”是指克，“μg”是指微克，并且“ng”是指纳克，“PCR”是指聚合酶链反应，“OD”是指光密度，“OD_{600}”是指在 600nm 波长下测量的光密度，“kDa”是指千道尔顿，“g”是指引力常数，“bp”是指碱基对，“kbp”是指千碱基对，“% w/v”是指重量 / 体积百分比，“% v/v”是指体积 / 体积百分比，“wt%”是指重量百分比，“HPLC”是指高效液相色谱，并且“GC”是指气相色谱。

[0157] 实施例中使用的标准重组 DNA 和分子克隆技术是本领域熟知的并且描述于 Sambrook, J., Fritsch, E.F. 和 Maniatis, T.Molecular Cloning :A Laboratory Manual ; Cold Spring Harbor Laboratory Press :Cold Spring Harbor, NY(1989)(Maniatis) 和 T.J.Silhavy, M.L.Bennan 和 L.W.Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.(1984) 以 及 Ausubel, F.M. 等人, Current Protocols in Molecular Biology, Greene Publishing Assoc. 和 Wiley-Interscience 出 版 (1987) 和 Methods in Yeast Genetics,2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY。

[0158] 适于细菌培养物维持和生长的材料和方法是本领域所熟知的。适用于以

下实施例的技术可见于Manual of Methods for General Bacteriology(Phillipp Gerhardt, R.G.E.Murray, Ralph N.Costilow, Eugene W.Nester, Willis A.Wood, Noel R.Krieg 和 G.Briggs Phillips 编辑), American Society for Microbiology, Washington, DC.(1994)) 或 Thomas D.Brock, Biotechnology :A Textbook of Industrial Microbiology, 第二版, Sinauer Associates, Inc., Sunderland, MA.(1989)。除非另外指明,使用的用于微生物细胞生长和维持的所有试剂、限制性酶和材料得自 Aldrich Chemicals(Milwaukee, WI)、BD Diagnostic Systems(Sparks, MD)、Life Technologies(Rockville, MD) 或 Sigma Chemical Company(St.Louis, MO)。除非另外指明,微生物菌株得自美国典型培养物保藏中心 (ATCC), Manassas, VA。表 3 给出了以下实施例中使用的寡核苷酸引物。所有寡核苷酸引物由 Sigma-Genosys(Woodlands, TX) Integrated DNA Technologies(Coralsville, IA) 或 Invitrogen Corp(Carlsbad, CA) 合成。

[0159] DNA 片段用 Qiaquick PCR 纯化试剂盒 (Qiagen Inc., Valencia, CA) 进行纯化。质粒 DNA 用 QIAprep Spin Miniprep 试剂盒 (Qiagen Inc., Valencia, CA) 制备。植物乳杆菌 (L.plantarum) PN0512 基因组 DNA 用 MasterPure DNA 纯化试剂盒 (Epicentre, Madison, WI) 制备。

[0160] 完全合成培养基描述于 Amberg, Burke 和 Strathern, 2005, Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 中。

[0161] 转化

[0162] 植物乳杆菌 (Lactobacillus plantarum) PN0512 通过以下方法转化:将 PN0512 细胞接种在 5mL 包含 1% 甘氨酸 (Sigma-Aldrich, St.Louis, MO) 的乳杆菌 MRS 培养基 (Accumedia, Neogen Corporation, Lansing, MI) 中,在 30℃下生长过夜。用 OD600 为 0.1 的过夜培养物接种于具有 1% 甘氨酸的 100mL 的 MRS 培养基,在 30℃下生长至 OD600 为 0.7。在 4℃下以 3700×g 离心 8 分钟收获细胞,用 100mL 冷的 1mM 的 $MgCl_2$(Sigma-Aldrich, St.Louis, MO) 洗涤,在 4℃下以 3700×g 离心 8 分钟,用 100mL 冷的 30% PEG-1000(Sigma-Aldrich, St.Louis, MO) 洗涤,在 4℃下以 3700×g 再离心 20 分钟,然后重悬在 1mL 冷的 30% PEG-1000 中。60μl 细胞与～ 100ng 质粒 DNA 在冷的 1mm 间隙电穿孔管中混合,并且用 BioRad Gene Pulser(Hercules, CA) 在 1.7kV, 25μF 和 400Ω 下电穿孔。将细胞重悬在 1mL 包含 500mM 的蔗糖 (Sigma-Aldrich, St.Louis, MO) 和 100mM 的 $MgCl_2$ 的 MRS 培养基中,在 30℃下培养 2 小时,置于包含 1μg/mL 或 2μg/mL 红霉素 (Sigma-Aldrich, St.Louis, MO) 的 MRS 培养基平板上,然后置于包含 Pack-Anaero 小袋 (Mitsubishi Gas Chemical Co., Tokyo, Japan) 的厌氧箱中并在 30℃下培养。

[0163] HPLC 方法

[0164] 对发酵副产物组成的分析是本领域的技术人员所熟知的。例如,一种使用 Shodex SH-1011 柱和 Shodex SH-G 保护柱 (二者均可得自 Waters Corporation, Milford, MA) 并联合折射率 (RI) 检测的高效液相色谱 (HPLC) 方法。用 0.01M 的 H_2SO_4 作为流动相,以 0.5mL/min 流量和 50℃的柱温实现色谱分离。异丁醇的保留时间为约 47.6 分钟。

[0165] 实施例 1

[0166] ilvD 整合载体和 PN0512ΔldhDΔldhL1::ilvDL1$^{\pm}$ 整合菌株的构建

[0167] 这个实施例描述了将乳酸乳球菌 (Lactococcus lactis) ilvD 基因整合进植物乳

杆菌 (L.plantarum) 菌株 PN0512ΔldhDΔldhL1 的染色体以表达 DHAD。在美国专利申请 #61/100786 的实施例 1 中描述了植物乳杆菌 (L.plantarum)PN0512ΔldhDΔldhL1 的构建。删除了这个菌株的两个编码主要乳酸脱氢酶 :ldhD 和 ldhL1 的基因。在植物乳杆菌 (Lactobacillus plantarum)PN0512(ATCC 菌株 #PTA-7727) 中进行了双删除。

[0168] 使用基于两步同源重组的方法构建基因敲除株以产生无标记的基因删除 (Ferain 等人,1994, J.Bact.176 :596)。所述方法利用穿梭载体 pFP996(SEQ ID NO110)。pFP996 是用于革兰氏阳性菌的穿梭载体。它既可在大肠杆菌中复制也可在革兰氏阳性菌中复制。它包含得自 pBR322(核苷酸 #2628 至 5323) 和 pE194(核苷酸 #43 至 2627) 的复制起点。pE194 是最初分离自革兰氏阳性菌金黄色葡萄球菌 (Staphylococcus aureus) 的小质粒 (Horinouchi 和 Weisblum J.Bacteriol.(1982)150(2) :804-814)。在 pFP996 中,多个克隆位点 (核苷酸 #1 至 50) 包含 EcoRI、BglII、XhoI、SmaI、ClaI、KpnI 和 HindIII 的限制性位点。有两个抗生素抗性标记 ;一个是抗氨苄青霉素标记,另一个是抗红霉素标记。为了选择目的,在大肠杆菌的转化中使用氨苄青霉素并且在植物乳杆菌 (L.plantarum) 中使用红霉素进行选择。

[0169] 将两个 DNA 片段 (每个包含 900bp 至 1200bp 的序列,其在拟删除序列的上游或下游) 克隆进质粒以提供两个遗传交换的同源性区域。细胞生长多代 (30-50 代) 以使交换事件发生。初始交换 (单交换) 通过同源重组,利用质粒上的两个同源区域中的一个将质粒整合进染色体中。二次交换 (双交换) 事件生成或者野生型序列或者拟删除的基因。导致初始整合事件的序列间交换将生成野生型序列,而其它同源区域之间的交换将生成期望的删除。通过抗生素敏感型筛选二次交换事件。通过 PCR 和 DNA 测序分析单交换和双交换事件。

[0170] ΔldhD

[0171] 用于删除 ldhD 基因的敲除盒通过从 PN0512 基因组 DNA 中扩增出上游侧接区域而生成,所述侧接区域具有引物 Top D F1(SEQ ID NO :111),其包含 EcoRI 位点和 Top D R1(SEQ ID NO :112)。利用包含 Xhol 位点的引物 Bot D F2(SEQ ID NO :114) 和 Bot D R2(SEQ ID NO :113) 扩增包括部分 ldhD 编码序列的下游同源区域。通过如下所述的 PCR SOE 接合两个同源区域。凝胶纯化 0.9kbp 的上游和下游 PCR 产物。在 PCR 反应中等量混合 PCR 产物并用引物 Top D F1 和 Bot D R2 进行再扩增。凝胶纯化最终的 1.8kbp 的 PCR 产物并将 TOPO 克隆进 pCR4BluntII-TOPO(Invitrogen) 以制备载体 pCRBluntII::ldhD。为了制备携带内部缺失 ldhD 基因的整合载体,pFP996 用 EcoRI 和 XhoI 消化并凝胶纯化 5311-bp 的片段。载体 pCRBluntII::ldhD 用 EcoRI 和 XhoI 消化并凝胶纯化 1.8kbp 的片段。ldhD 敲除盒和载体用 T4DNA 连接酶连接,得到载体 pFP996::ldhDko。

[0172] 制备电感受态植物乳杆菌 (Lactobacillus plantarum)PN0512 细胞,用 pFP996::ldhD ko 转化并将其置于包含 1μg/mL 的红霉素的 MRS 上。为了得到单交换事件 (sco),转化子在 37℃下 MRS 培养基中传代大约 50 代。在生长后,将等分试样接种到包含 1μg/mL 红霉素的 MRS 平板上以获得单菌落。抗红霉素菌落通过利用引物 ldhD Seq F1(SEQ ID NO :115) 和 D check R(SEQ ID NO :116) 的 PCR 扩增进行筛选以分辨野生型和携带 sco 事件的克隆。为了获得具有双交换的克隆,将 sco 菌株在 37℃下在具有 20mM 的 D,L- 乳酸 (Sigma,St.Louis,MO) 的 MRS 培养基中传代大约 30 代,然后接种到带乳酸盐的 MRS 平板上

以获得单菌落。挑取菌落并涂布在包含乳酸盐的MRS和具有包含1μg/mL红霉素的乳酸盐的MRS上以寻找对红霉素敏感的菌落。敏感型菌落通过利用引物D check R(SEQ ID NO:116)和D check F3(SEQ ID NO:1117)的PCR扩增来筛选。野生型菌落提供3.2kbp的产物并且称为PN0512ΔldhD的删除克隆提供2.3kbp的PCR产物。

[0173] ΔldhDΔldhL1

[0174] 为了制备ΔldhL1ΔldhD双删除菌株，在PN0512ΔldhD菌株背景中删除ldhL1基因。用于删除ldhL1基因的敲除盒从PN0512基因组DNA中扩增出来。ldhL1左同源臂利用包含BglII限制性位点的引物oBP31(SEQ ID NO:118)和包含XhoI限制性位点的引物oBP32(SEQ ID NO:119)进行扩增。ldhL1右同源臂利用包含XhoI限制性位点的引物oBP33(SEQ ID NO:120)和包含XmaI限制性位点的引物oBP34(SEQ ID NO:121)进行扩增。将ldhL1左同源臂克隆进BglII/XhoI位点中并将ldhL1右同源臂克隆进XhoI/XmaI位点中，上述位点均属于pFP996pyrFΔerm，它是pFP996的衍生物。pFP996pyrFΔerm包含pyrF序列(SEQ ID NO:122)，该序列编码植物乳杆菌(Lactobacillus plantarum)PN0512的乳清酸核苷-5′-磷酸脱羧酶，代替pFP996中的红霉素编码区。质粒携带的pyrF基因与ΔpyrF菌株中的化学5-氟乳清酸一起可用作用于分离二次同源交换的有效反选择方法。在用XmaI消化后分离包含ldhL1同源臂的Xmal片段并将其克隆进pFP996的XmaI限制性位点中，生成900bp的左同源区和1200bp的右同源区，得到载体pFP996-ldhL1-arms。

[0175] PN0512ΔldhD用pFP996-ldhL1-arms转化并在30℃下在含有乳酸盐(20mM)和红霉素(1μg/mL)的乳杆菌MRS培养基中生长大约10代。然后在将培养物置于包含乳酸盐和红霉素(1μg/mL)的MRS上之前，通过将转化子连续接种在MRS+乳酸盐上使其在非选择性条件下，在37℃生长大约50代。通过用于单交换的菌落PCR筛选分离物，所述单交换使用染色体特异性引物oBP49(SEQ ID NO:123)和质粒特异性引物oBP42(SEQ ID NO:124)。在将培养物置于包含乳酸盐的MRS培养基上之前，通过在非选择性条件下连续接种于包含乳酸盐的MRS中使单交换整合子在37℃下生长大约40代。将分离物涂布在包含乳酸盐的MRS平板上，在37℃下生长，然后涂板在包含乳酸盐和红霉素(1μg/mL)的MRS平板上。通过使用染色体特异性引物oBP49(SEQ ID NO:123)和oBP56(SEQ ID NO:125)的菌落PCR筛选红霉素敏感型分离物以确定野生型或二次交换删除的存在。野生型序列生成3505bp的产物，并且删除序列生成2545bp的产物。通过PCR产物测序确认删除并通过利用引物oBP42(SEQ ID NO:124)和oBP57(SEQ ID NO:126)的菌落PCR测试质粒的删除。

[0176] 植物乳杆菌(Lactobacillus plantarum)PN0512 ldhDldhL1双删除菌株称为PNP0001。ΔldhD删除包括83bp的上游片段，其中ldhD起始密码子为332的氨基酸279。ΔldhL1删除包括包含最后氨基酸的fMet。

[0177] 构建从ldhL1启动子开始表达的乳酸乳球菌(L.lactis)ilvD编码区的单拷贝染色体整合，所述整合如上所述通过相同的两步同源重组方法以产生无标记的整合，该方法使用pFP996穿梭载体，不同的是二次交换事件产生野生型序列或期望的整合而不是删除。将包含期望整合位点的序列上游和下游的两片段DNA克隆进质粒中以提供两个遗传交换的同源区域。

[0178] 设计两个DNA片段(同源臂)以提供两个遗传交换的同源区域，使得整合将位于菌株PN0512ΔldhDΔldhL1中的ldhL1启动子的ilvD编码区下游。克隆到质粒中的左和

右同源臂每个为大约 1200 个碱基对。左同源臂从植物乳杆菌 (L.plantarum)PN0512 基因组 DNA 中，使用包含 BglII 限制性位点的引物 oBP31(SEQ ID NO:118) 和包含 XhoI 限制性位点的引物 oBP32(SEQ ID NO 119)，利用 Phusion High-Fidelity PCR Master Mix 进行扩增。右同源臂从植物乳杆菌 (L.plantarum)PN0512 基因组 DNA 中，使用包含 XhoI 限制性位点的引物 oBP33(SEQ ID NO:120) 和包含 XmaI 限制性位点的引物 oBP34(SEQ ID NO:121)，利用 Phusion High-Fidelity PCR Master Mix 进行扩增。左同源臂用 BglII 和 XhoI 消化，并且右同源臂用 XhoI 和 XmaI 消化。在用合适的限制性酶消化后，两个同源臂用 T4DNA 连接酶连接到相应的限制性位点 pFP996 中以生成载体 pFP996-ldhL1arms。

[0179] 从 pDM20-ilvD(乳酸乳球菌 (L.lactis))(SEQ ID NO:128) 中扩增包含来自乳酸乳球菌 (Lactococcus lactis)(SEQ ID NO:94) 的 ilvD 编码区和核糖体结合序列 (RBS;SEQ ID NO:127) 的 DNA 片段。pDM20-ilvD(乳酸乳球菌 (L.lactis)) 的构建在以引用方式并入本文的美国专利申请 #61/100809 中进行了描述。这个质粒是包含 ilvD 编码区的 pDM20，其通过 PCR 得自乳酸乳球菌乳脂亚种 (L.lactis subsp lactis)NCDO2118(NCIMB 702118)[Godon 等人，J.Bacteriol.(1992)174:6580-6589]，并且该质粒包含加到 5”PCR 引物中的核糖体结合序列 (SEQ ID NO:1127)。pDM20 是经修饰的 pDM1(SEQ ID NO:129)，其包含最小的 pLF1 复制子 (～ 0.7kbp) 和得自植物乳杆菌 (Lactobacillus plantarum)ATCC14917 质粒 pLF1 的 pemK-pemI toxin-antitoxin(TA)、来自 pACYC184 的 P15A 复制子、用于在大肠杆菌和植物乳杆菌 (L.plantarum) 中进行选择的抗氯霉素标记、以及 P30 合成启动子 [Rud 等人，Microbiology(2006)152:1011-1019]。通过删除包括 lacZ 区域的核苷酸 3281-3646 修饰载体 pDM1，所述 lacZ 区域被多克隆位点取代。引物 oBP120(SEQ ID NO:1130) 包含 XhoI 位点，并且 oBP182(SEQ ID NO:131) 包含 DrdI、PstI、HindIII 和 BamHI 位点，它们用于利用 Phusion High-Fidelity PCR Master Mix 扩增来自 pDM1 的 P30 启动子。所得 PCR 产物和 pDM1 载体用 XhoI 和 DrdI 消化，得到 lacZ 和 P30。连接 PCR 产物和 pDM1 消化大片段以生成载体 pDM20，其中再插入 P30 启动子，其由 XhoI 和 DrdI 限制性位点界定。

[0180] 包含 ilvD 编码区和 RBS(SEQ ID NO:132) 的 DNA 片段通过 PCR 获得，所述 PCR 用 pDM20-ilvD(乳酸乳球菌 (L.lactis)) 作为模板，使用包含 XhoI 限制性位点的引物 oBP246(SEQ ID NO:133) 和包含 XhoI 限制性位点的引物 oBP237(SEQ ID NO:134)，利用 Phusion High-Fidelity PCR Master Mix 进行。在用 XhoI 消化后，所得 PCR 产物和 pFP996-ldhL1arms 用 T4 DNA 连接酶连接。通过 PCR 筛选具有与左同源臂中的 ldhL1 启动子相同取向的插入序列的克隆，该 PCR 使用载体特异性引物 oBP57(SEQ ID NO:126) 和 ilvD- 特异性引物 oBP237(SEQ ID NO:134)。具有正确取向的插入序列的克隆称为 pFP996-ldhL1arms-ilvDL1。

[0181] 通过用 pFP996-ldhL1arms-ilvDL1 转化植物乳杆菌 (L.plantarum)PN0512ΔldhDΔldhL1 来整合乳酸乳球菌 (L.lactis)ilvD 编码区。用 PN0512ΔldhDΔldhL1 接种在 5mL 包含 0.5％甘氨酸 (Sigma-Aldrich，St.Louis，MO) 的乳杆菌 MRS 培养基 (Accumedia，Neogen Corporation，Lansing，MI) 中，在 30℃下生长过夜。用 OD600 为 0.1 的过夜培养物接种于 100mL 具有 0.5％甘氨酸的 MRS 培养基，在 30℃下生长至 OD600 为 0.7。在 4℃下以 3700×g 离心 8 分钟收获细胞，用 100mL 冷的 1mM 的 $MgCl_2$(Sigma-Aldrich，St.Louis，MO) 洗涤，在 4℃下以 3700×g 离心 8 分钟，用 100mL 冷的

30％ PEG-1000(Sigma-Aldrich,St.Louis,MO) 洗涤，在 4℃下以 3700×g 再离心 20 分钟，然后重悬在 1mL 冷的 30％ PEG-1000 中。60μl 细胞与～ 100ng 质粒 DNA 在冷的 1mm 间隙电穿孔管中混合，并且用 BioRad Gene Pulser(Hercules，CA) 在 1.7kV，25μF 和 400Ω 下电穿孔。将细胞重悬在 1mL 包含 500mM 蔗糖 (Sigma-Aldrich，St.Louis，MO) 和 100mM 的 $MgCl_2$ 的 MRS 培养基中，在 30℃下培养 2 小时，并且置于包含 2μg/mL 红霉素 (Sigma-Aldrich，St.Louis，MO) 的 MRS 培养基平板上。

[0182] 通过使用 ilvD 特异性引物 oBP237(SEQ ID NO：134) 和 oBP246(SEQ ID NO：133) 的 PCR 筛选转化子。转化子在 30℃下在包含红霉素 (1μg/mL) 的乳杆菌 MRS 培养基中生长大约 8 代，然后在 37℃下通过连续接种在乳杆菌 MRS 培养基中生长大约 40 代。将培养物置于包含红霉素 (0.5μg/mL) 的乳杆菌 MRS 培养基上。通过菌落 PCR 筛选分离物以获得单交换，所述 PCR 使用染色体特异性引物 oBP49(SEQ ID NO：123) 和质粒特异性引物 oBP42(SEQ ID NO：124)。

[0183] 通过连续接种在乳杆菌 MRS 培养基中，单交换整合子在 37℃下生长大约 43 代。将培养物置于 MRS 培养基上。将菌落涂布接种到 MRS 平板上并在 37℃下生长。然后将分离物涂布接种到包含红霉素 (0.5μg/mL) 的 MRS 培养基上。通过使用染色体特异性引物 oBP49(SEQ ID NO：123) 和 oBP56(SEQ ID NO：125) 的 (菌落)PCR 筛选红霉素敏感型分离物以确定野生型或二次交换整合的存在。野生型序列生成 2600bp 的产物，并且整合序列生成 4300bp 的产物。通过对 PCR 产物测序来确认整合，并且将鉴定的整合菌株称为 PN0512ΔldhDΔldhL1::ilvDL1^{+}。

[0184] 实施例 2

[0185] suf 操纵子启动子整合载体和 PN0512ΔldhDΔldhL1::ilvDL1^{+}suf::P5P4^{+} 整合菌株的构建

[0186] 这个实施例描述了将两个启动子整合进植物乳杆菌 (L.plantarum) PN0512ΔldhDΔldhL1::ilvDL1^{+} 的染色体中。将启动子整合到 suf 操纵子的上游，其基因产物用于铁硫簇装配。启动子整合产生内源铁硫簇机构表达增加的菌株。

[0187] 通过两步同源重组方法构建 suf 操纵子染色体启动子整合以产生无标记的整合，所述方法使用如上所述的穿梭载体 pFP996(SEQ ID NO：110)。

[0188] suf 操纵子启动子整合载体通过三个步骤构建。在第一步骤中，将包含 suf 操纵子 (sufC 和部分 sufD)5′ 部分的右同源臂片段克隆进 pFP996。在第二步骤中，将合成启动子 P5 和 P4[Rud 等人，Microbiology(2006)152：1011] 克隆进右臂的 pFP996- 右臂克隆上游。在最终步骤中，将包含 feoBA 操纵子中的天然 suf 启动子和上游序列的左同源臂片段克隆进 P5P4 启动子的 pFP996-P5P4- 右臂克隆上游。

[0189] 通过 PCR 从植物乳杆菌 (L.plantarum)PN0512 基因组 DNA 中扩增右同源臂 DNA 片段 (SEQ ID NO：135)，所述 PCR 使用包含 XmaI 限制性位点的引物 AA199(SEQ ID NO：136) 和包含 KpnI 限制性位点的引物 AA200(SEQ ID NO：137)，利用 Phusion High-Fidelity PCR Master Mix 进行。在用 XmaI 和 KpnI 消化后利用 T4DNA 连接酶连接右同源臂 PCR 片段和 pFP996 以生成 pFP996-sufCD。通过用两个部分互补的引物序列进行 PCR 生成包含启动子 P5 和 P4 的 DNA 片段。引物 AA203(SEQ ID NO：138) 包含 XhoI 位点、P5 启动子序列和部分 P4 启动子序列，将其与包含 XmaI 位点和 P4 启动子序列的引物 AA204(SEQ ID NO：139) 组

合使用，并且用 Phusion High-Fidelity PCR Master Mix 进行 PCR。所得 PCR 产物然后用引物 AA206(SEQ ID NO:140) 和 AA207(SEQ ID NO:141)，利用 Phusion High-Fidelity PCR Master Mix 进行扩增。在用 XhoI 和 XmaI 消化后连接 P5P4PCR 产物和 pFP996-sufCD 以生成 pFP996-P5P4-sufCD。通过从植物乳杆菌 (L.plantarum)PN0512 基因组 DNA 中扩增左同源臂 DNA 片段 (SEQ ID NO:142)，所述扩增使用包含 EcoRI 限制性位点的引物 AA201(SEQ ID NO:143) 和包含 XhoI 限制性位点的引物 AA202(SEQ ID NO:144)，利用 Phusion High-Fidelity PCR Master Mix 进行。在用 EcoRI 和 XhoI 消化后用 T4DNA 连接酶连接左同源臂和 pFP996-P5P4-sufCD 以生成 pFP996-feoBA-P5P4-sufCD。通过测序确认载体。所述载体具有五个碱基对删除 (TTGTT)，包括在上游 P5 启动子中的部分 -35 六聚物。

[0190] suf 操纵子的合成启动子 (P5P4) 上游的整合通过如上所述用 pFP996-feoBA-P5P4-sufCD 转化植物乳杆菌 (L.plantarum) PN0512ΔldhDΔldhL1::ilvDL1^{+} 来完成。转化子在 30℃下在包含红霉素 (2μg/mL) 的乳杆菌 MRS 培养基中生长大约 20 代。将培养物置于包含红霉素 (0.5μg/mL) 的乳杆菌 MRS 培养基上。通过用于单交换的菌落 PCR 筛选分离物，所述单交换使用染色体特异性引物 AA209(SEQ ID NO:145) 和质粒特异性引物 AA210(SEQ ID NO:146)。通过连续接种在乳杆菌 MRS 培养基中，单交换整合子在 37℃下生长大约 30 代。将培养物置于 MRS 培养基上。筛选对红霉素敏感的分离物。通过使用 P5 特异性引物 AA211(SEQ ID NO:147) 和染色体特异性引物 oBP126(SEQ ID NO:148) 的 (菌落)PCR 筛选分离物以确定野生型或二次交换整合的存在。将鉴定的整合菌株称为 PN0512ΔldhDΔldhL1::ilvDL1^{+}suf::P5P4^{+}。

[0191] 实施例 3

[0192] Tn5- 转座子载体 (pTN6) 的构建及其在 PgroE-kivD(o)-sadB(o) 盒整合中的应用

[0193] Tn5 是细菌转座子，其已经在大肠杆菌中进行了充分的表征 (Johnson & Reznikoff,Nature(1983)304:280-282)。用于乳酸菌 (LAB) 的 Tn5 介导的转座体系在以引用方式并入本文的美国临时专利申请 61/246717 中进行了描述。在这个实施例中，开发了 Tn5- 转座子载体用作随机基因整合到 LAB 染色体中的递送体系。开发的 Tn5- 转座子载体 (pTN6)(SEQ ID NO:149) 是大肠杆菌 - 植物乳杆菌 (L.plantarum) 穿梭载体。质粒 pTN6 包含转座酶基因 (tnp)、转座酶识别核苷酸序列 Tn5IE(末端内的 19 个碱基对) 和 Tn5OE(末端外的 19 个碱基对)、两个抗生素抗性标记；一个是氯霉素抗性标记，并且另一个是红霉素抗性标记、大肠杆菌 P15A 复制起点、温度敏感型的植物乳杆菌 (L.plantarum)pE194 复制起点 (Horinouchi 和 Weisblum J.Bacteriol.(1982)150:804-814)、以及两个 loxP 核苷酸序列 (34 个碱基对)。氯霉素抗性基因侧接用于随后的 Cre 重组酶切除的 loxP 位点。多克隆位点 (MSC) 包含 BamHI、NotI、ScaI 和 SpeI 限制性位点，其位于 loxP 和 Tn5OE 位点之间。氯霉素抗性基因、两个 loxP 位点和 MCS 侧接 Tn5IE 和 Tn5OE。

[0194] 为了构建 Tn5- 转座子载体 pTN6，首先由 Genscript Corp(Piscataway,NJ) 合成 1,048bp 的 Tn5IE-loxP-cm-loxP 盒，其包含 Tn5IE、loxP、氯霉素抗性基因 (cm) 和 loxP(SEQ ID NO:150)。将 Tn5IE-loxP-cm-Pspac-loxP 盒克隆进 pUC57 载体中 (Genscript Corp,Piscataway,NJ)，生成质粒 pUC57-Tn5IE-loxP-cm-loxP。在 spac 启动子的控制下表达氯霉素抗性基因 (Yansura & Henner,(1984)Proc Natl Acad Sci USA.81:439-443) 以在大肠杆菌和植物乳杆菌 (L.plantarum) 中选择。用 NsiI 和 SacI 消化质粒

pUC57-Tn5IE-loxP-cm-loxP 并凝胶纯化 1,044bp 的 Tn5IE-loxP-cm-loxP 片段。用 NsiI 和 SacI 消化质粒 pFP996(SEQ ID NO:110) 并凝胶纯化 4,417bp 的 pFP996 片段，其包含 pBR322 和 pE194 复制起点。Tn5IE-loxP-cm-loxP 片段与 4,417bp 的 pFP996 片段连接以生成 pTnCm。

[0195] 其次，用 P15A 复制起点取代 pTnCm 上的 pBR322 复制起点。用 AatII 和 SalI 消化质粒 pTnCm 并凝胶纯化 2,524bp 的 pTnCm 片段，其包含 pE194 复制起点和 Tn5IE-loxP-cm-loxP 盒。913bp 的 p15A 复制起点利用 PCR 从 pACYC184[Chang 和 Cohen, J. Bacteriol. (1978) 134 : 1141-1156] 中扩增出来，所述 PCR 使用包含 SalI 限制性位点和 19bp 的 Tn5OE 核苷酸序列的引物 T-P15A(SalITn5OE)(SEQ ID NO:151) 以及包含 AatII 限制性位点的引物 B-P15A(AatII)(SEQ ID NO:152)，并且利用 Phusion High-Fidelity PCR Master Mix(New England Biolabs, Ipswich, MA)。在用 SalI 和 AatII 限制性酶消化后，P15A 片段与 2,524bp 的 pTnCm 片段连接以生成 pTN5。

[0196] 第三，将抗红霉素基因 (erm) 克隆进 pTN5 上的 HindIII 位点。通过 PCR 扩增从载体 pFP996(SEQ ID NO:110) 中生成 1,132bp 的抗红霉素基因 (erm)DNA 片段，所述 PCR 使用包含 NsiI 限制性位点的引物 T-erm(HindIII)(SEQ ID NO:153) 和包含 NsiI 限制性位点的 B-erm(HindIII)(SEQ ID NO:154)，利用 Phusion High-Fidelity PCR Master Mix，并且将上述片段克隆进 pTN5 上的 HindIII 限制性位点，生成 pTN5-erm。

[0197] 最后，将编码转座酶的 tnp 基因序列融合到 npr(来自解淀粉芽孢杆菌 (Bacillus amyloliquefaciens) 的中性蛋白酶) 启动子中 [Nagarajan 等人, J. Bacteriol (1984) 159 : 811-819]，这通过 SOE(重叠延伸拼接)PCR 完成并将其克隆进 pTN5-erm 上的 NsiI 位点。包含 Pnpr 启动子 (SEQ ID NO:155) 的 DNA 片段通过 PCR 从 pBE83[Nagarajan 等人, Appl Environ Microbiol (1993) 59 :3894-3898] 中扩增出来，所述 PCR 使用包含 NsiI 限制性位点的 T-Pnpr(NsiI)(SEQ ID NO:156) 和包含 17bp 重叠序列的 B-Pnpr(tnp)(SEQ ID NO:157) 的引物组，利用 Phusion High-Fidelity PCR Master Mix。tnp 编码区 (SEQ ID NO:108) 通过 PCR 从 pUTmTn5-(Sharpe 等人, Appl Environ Microbiol (2007) 73 :1721-1728) 中扩增出来，所述 PCR 使用包含 21bp 重叠序列的 T-tnp(Pnpr)(SEQ ID NO:1158) 和包含 NsiI 限制性位点的 B-tnp(NsiI)(SEQ ID NO:159) 的引物组，利用 Phusion High-Fidelity PCR Master Mix。混合两个反应的 PCR 产物并使用外引物 (T-Pnpr(NsiI) 和 B-tnp(NsiI)) 进行扩增，结果得到 Pnpr-tnp 融合 DNA 片段 (SEQ ID NO:160)。质粒 pTN5-erm 用 NsiI 消化并用 Calf 肠磷酸酶 (New England Biolabs, MA) 处理以防止自身连接。消化的 pTN5-erm 载体与用 NsiI 消化的 Pnpr-tnp 片段连接。通过电穿孔将连接混合物转化到大肠杆菌 Top10 细胞 (Invitrogen Corp, Carlsbad, CA) 中。在包含 25μg/mL 氯霉素的 LB 平板上，在 37℃下选择转化子。然后通过菌落 PCR，使用 Pnpr-tnp 盒的外引物筛选转化子，并且通过用引物 pTnCm(711)(SEQ ID NO:161)、pTnCm(1422)(SEQ ID NO:162) 和 pTnCm(3025)(SEQ ID NO:163) 进行 DNA 测序来确认。所得质粒称为 pTN6。

[0198] 将这个 Tn5- 转座子载体 pTN6 用作将 PgroE-kivD(o)-sadB(o) 盒整合到 PN0512ΔldhDΔldhL1::ilvDL1^{+}suf::P5P4^{+} 菌株的染色体中的随机基因递送体系。包含 PgroE 启动子 (Yuan 和 Wong, J. Bacteriol (1995) 177 :5427-5433)(SEQ ID NO:164) 的 DNA 片段通过 PCR 从枯草芽孢杆菌 (Bacillus subtilis) 的基因组 DNA 中扩增出来，所述 PCR 使用包含 SalI 和 KpnI 限制性位点的 T-groE(SalIKpnI)(SEQ ID NO:165) 和包含 BamHI 限制性位点

的 B-groE(BamHI)(SEQ ID NO:166) 的引物组，利用 Phusion High-Fidelity PCR Master Mix。在用 SalI 和 BamHI 限制性酶消化后所得 154bp 的 PgroE 启动子片段被克隆进质粒 pTN6 的 SalI 和 BamHI 位点中，生成 pTN6-PgroE。编码来自乳酸乳球菌 (Lactococcus lactis) 的支链酮酸脱羧酶的 kivD 基因的编码区经密码子优化以在植物乳杆菌 (L.plantarum) 中表达。经优化的编码区序列称为 kivD(o)(SEQ ID NO:90)，其具有 RBS，由 Genscript Corp(Piscataway,NJ) 合成。将 kivD(o) 编码区连同 RBS(SEQ ID NO:167) 一起克隆进 pUC57 载体中，生成质粒 pUC57-kivD(o)。质粒 pUC57-kivD(o) 用 BamHI 和 NotI 消化，并且凝胶纯化 1,647bp 的 RBS-kivD(o) 片段。将 RBS-kivD(o) 片段克隆进 pTN6-PgroE 上的 BamHI 和 NotI 限制性位点，生成 pTN6-PgroE-kivD(o)。通过菌落 PCR 确认正确的克隆，所述 PCR 使用引物 T-groE(SalIKpnI) 和 kivD(o)R(SEQ ID NO:165 和 168)，生成期望大小的 1,822bp 片段。然后如美国专利申请 #12/430356 所述，将来自木糖氧化无色杆菌 (Achromobacter xylosoxidans) 的支链醇脱氢酶的 sadB 基因编码区克隆到 pTN6-PgroE-kivD(o) 的 kivD(o) 编码区下游。木糖氧化无色杆菌 (A.xylosoxidans) 的 sadB 编码区经密码子优化以在植物乳杆菌 (L.plantarum) 中表达。新编码区称为 sadB(o)(SEQ ID NO:105)，其具有 RBS，由 Genscript Corp(Piscataway, NJ) 合成并被克隆到 pUC57 载体中生成质粒 pUC57-sadB(o)。包含 RBS 和 sadB(o) 编码区的 1,089bp 的 DNA 片段 (SEQ ID NO:169) 通过 PCR 从 pUC57-sadB(o) 中扩增出来，所述 PCR 使用包含 NotI 限制性位点的 T-sadB(o)(NotI)(SEQ ID NO:170) 和包含 NotI 限制性位点的 B-sadB(o)(NotI)(SEQ ID NO:171) 的引物组，利用 Phusion High-Fidelity PCR Master Mix。在用 NotI 消化后，将 RBS-sadB(o) 基因片段克隆进 pTN6-PgroE-kivD(o) 的 NotI 限制性位点中，生成 pTN6-PgroE-kivD(o)-sadB(o)。通过使用 kivD(o)1529(SEQ ID NO:172) 和 B-spac(cm)(SEQ ID NO:173) 引物的 DNA 测序确认正确的克隆。在这个构建过程中，sadB(o) 和 kivD(o) 编码区在操纵子中从 PgroE 启动子开始表达。

[0199] 如一般方法所述，通过电穿孔将所得质粒 pTN6-PgroE-kivD(o)-sadB(o) 转化到 PN0512ΔldhDΔldhL1::ilvDL1^{+}suf::P5P4^{+} 中。在补充有 7.5g/mL 氯霉素的乳杆菌 MRS 培养基上选择转化子。抗氯霉素菌落在包含 7.5μg/mL 氯霉素的乳杆菌 MRS 培养基上，在 30℃的许可温度下生长大约 10 代。将 1/100 稀释的培养物接种到新鲜 MRS 培养基上，并且通过连续接种到乳杆菌 MRS 培养基上使其在 37℃下生长大约 20 代。将培养物置于含有 7.5μg/mL 氯霉素的乳杆菌 MRS 上。通过将菌落再次划线接种到包含 1.5μg/mL 红霉素的乳杆菌 MRS 平板上筛选分离物，所述平板用于红霉素敏感型菌落，推测其包含染色体整合的 PgroE-kivD(o)-sadB(o) 盒以及转座子。通过使用 kivD(o) 序列特异性引物 KivD(o)1529 和 sadB(o) 序列特异性引物 B-sadB(o)(NotI) 的菌落 PCR 确认转座子介导的整合子，从而制备期望大小的 PCR 产物 (1,220bp)。

[0200] 为了切除侧接染色体 loxP 位点的氯霉素抗性标记，根据一般方法中所述的规程将表达 Cre 重组酶的辅助质粒 pFP352(SEQ ID NO:174) 转化到转座子介导的整合子中并在 30℃下，在包含 1.5μg/mL 红霉素的乳杆菌 MRS 平板上生长。cre 重组酶通过 loxP 位点间的重组事件从染色体中切除氯霉素标记。将抗红霉素的转化子接种到 MRS 培养基中并在 37℃下生长大约 10 代。将培养物置于无抗生素的乳杆菌 MRS 上并在 30℃下生长。通过分别测试在包含 1.5μg/mL 红霉素的乳杆菌 MRS 平板上和在包含氯霉素 (7.5μg/mL) 的乳杆

菌MRS平板上的菌落生长筛选分离物中的红霉素和氯霉素敏感型菌落，从而验证pFP352的丢失和氯霉素标记的移除。最后，通过利用引物B-groE(BamHI)的基因组DNA测序确认整合子。使用MasterPure DNA Purification®试剂盒(Enpicentre,Inc.,Madison,WI)制备基因组DNA。DNA测序结果指示PgroE-kivD(o)-sadB(o)盒被插入编码糖原分支酶的glgB基因的编码区内，该酶催化1,4-α-D-葡聚糖链片段转化成相似葡聚糖链中的伯醇羟基。所得整合子称为PN0512ΔIdhDΔldhL1::ilvD(L1)suf::P5P4^{+}glgB::Tn5-PgroE-kivD(o)-sadB(o)。

[0201] 实施例4

[0202] pDM5-PldhL1-ilvC(乳酸乳球菌(L.lactis))载体的构建

[0203] 这个实施例的目的是描述将来自乳酸乳球菌乳脂亚种(Lactococcus lactis subsp lactis)NCDO2118(NCIMB 702118)的酮醇酸还原异构酶的ilvC编码区(SEQ ID NO:67)[Godon等人，J.Bacteriol.(1992)174:6580-6589]克隆进pDM5载体。

[0204] 质粒pDM5(SEQ ID NO:175)通过用融合到lacO操纵子序列和lacI阻遏蛋白基因上的枯草芽孢杆菌(B.subtilis)groE启动子(PgroE)取代pDM1的P30启动子进行构建。质粒pDM1在实施例1中进行了描述。质粒pHTO1(Mo Bi Tec，Goettingen，Germany)用SacI消化并用Klenow片段处理以制备平末端，用BamHI消化，然后凝胶纯化1,548bp的lacI-PgroE/lacO片段(SEQ ID NO:176)。将lacI-PgroE/lacO片段克隆进pDM1的KpnI(用Klenow片段处理得到平末端)和BamHI位点以取代P30启动子，生成pDM5。

[0205] DNA片段PldhL1-ilvC(乳酸乳球菌(L.lactis))包含ldhL1(得自植物乳杆菌(Lactobacillus plantarum)PN0512的L-乳酸脱氢酶)启动子(PldhL1)和得自乳酸乳球菌乳脂亚种(Lactococcus lactis subsp lactis)NCDO2118的ilvC编码区，该片段通过SOE(重叠延伸拼接)PCR生成。包含PldhL1启动子的DNA片段通过PCR从植物乳杆菌(Lactobacillus plantarum)PN0512的基因组DNA中扩增出来，所述PCR使用包含NotI限制性位点的T-ldhL1(NotI)(SEQ ID NO:177)和包含19bp重叠序列的B-ldhLI(CLI)(SEQ ID NO:178)的引物组，利用Phusion High-Fidelity PCR Master Mix。ilvC编码区通过PCR从乳酸乳球菌乳脂亚种(Lactococcus lactis subsp lactis)NCDO2118的基因组DNA中扩增出来，所述PCR使用包含17bp重叠序列的T-CLI(ldh)(SEQ ID NO:179)和包含PvuI限制性位点的B-CLI(PvuI)(SEQ ID NO:180)的引物组，利用Phusion High-Fidelity PCR Master Mix。混合两个片段的PCR产物并使用外引物T-ldhL1(NotI)和B-CLI(PvuI)进行扩增，结果得到PldhL1-ilvC(乳酸乳球菌(L.lactis))融合DNA片段。质粒pDM5用NotI和PvuI限制性酶消化，并且在用NotI和PvuI限制性酶消化后与PldhL1-ilvC(乳酸乳球菌(L.lactis))盒连接。通过电穿孔将连接混合物转化到大肠杆菌Top10细胞(Invitrogen Corp，Carlsbad，CA)中。在包含25μg/mL氯霉素的LB平板上，在37℃下选择转化子。然后通过菌落PCR，使用PldhL1-ilvC(乳酸乳球菌(L.lactis))盒的外引物筛选转化子，并且通过用T-ldhL1(NotI)(SEQ ID NO:177)和pDM(R)new(SEQ ID NO:181)进行DNA测序来确认。所得质粒称为pDM5-PldhL1-ilvC(乳酸乳球菌(L.lactis))(SEQ ID NO:182)。

[0206] 实施例5

[0207] pDM5-PldhL1-ilvC(荧光假单胞菌(P.fluorescence)5)载体的构建

[0208] 这个实施例的目的在于描述将来自荧光假单胞菌(Pseudomonas fluorescens)

PF5 的酮醇酸还原异构酶的 ilvC 编码区克隆进表达载体。

[0209] 荧光假单胞菌 (P. fluorescens)PF5 ilvC编码区经密码子优化以在作为首要宿主的植物乳杆菌 (Lactobacillus plantarum) 中表达。这个优化的编码区称为 ilvC(荧光假单胞菌 (P. fluorescens)PF5) 或 ilvC(Pf5),它与 RBS(SEQ ID NO :183) 一起由 Genscript Corp(Piscataway, NJ) 合成。编码区 ilvC(荧光假单胞菌 (P. fluorescens)PF5) 和 RBS 片段被克隆进 pUC57 载体中,生成质粒 pUC57-ilvC(荧光假单胞菌 (P. fluorescens) PF5),然后将其亚克隆进 pFP996-PldhL1(SEQ ID NO :184) 中作为 XhoI-KpnI 片段以生成 pFP996-PldhL1-ilvC(荧光假单胞菌 (P. fluorescens)PF5)。pFP996-PldhL1 包含如实施例 4 所述的 PldhL1 DNA 片段。PldhL1-ilvC(荧光假单胞菌 (P. fluorescens)PF5)DNA 片段 (SEQ ID NO :185) 包含 ldhL1(得自植物乳杆菌 (Lactobacillus plantarum)PN0512 的 L- 乳酸脱氢酶) 启动子 (PldhL1) 和得自荧光假单胞菌 (Pseudomonas fluorescens)PF5 的 ilvC 编码区,该片段通过 PCR 从 pFP996-PldhL1-ilvC(荧光假单胞菌 (P. fluorescens) PF5) 中生成,所述 PCR 使用包含 NotI 限制性位点的 T-ldhL1(NotI)(SEQ ID NO :186) 和包含 EcoRV 限制性位点的 B-CPf(o)(EcoRV)(SEQ ID NO :187) 的引物组。1,297bp 的 PldhL1-ilvC(荧光假单胞菌 (P. fluorescens)PF5) 片段用 NotI 和 EcoRV 进行消化并被连接到 pDM1-ilvD(乳酸乳球菌 (L. lactis)) 的 NotI 和 PvuII 位点中,生成 pDM1-ilvD(乳酸乳球菌 (L. lactis))-PldhL1-ilvC(荧光假单胞菌 (P. fluorescens)PF5)。在下文的美国临时专利申请 #61/100810 的实施例 1 中描述了 pDM1-ilvD(乳酸乳球菌 (L. lactis)) 的构建。

[0210] 用引物 3T-ilvDLI(BamHI)(SEQ ID NO :188) 和 5B-ilvDLI(NotI)(SEQ ID NO :189) 从乳酸乳球菌乳脂亚种 NCDO2118 的基因组 DNA 中 PCR 扩增了乳酸乳球菌 (Lactococcus lactis)ilvD 编码区 (SEQ ID NO :94)。用 Puregene Gentra 试剂盒 (QIAGEN ;Valencia, CA) 制备乳酸乳球菌乳脂亚种 (Lactococcus lactis subsp lactis)NCDO2118 基因组 DNA。用 NotI 消化了 1.7kbp 的乳酸乳球菌 ilvD PCR 产物 (ilvDLI) 并用 DNA 聚合酶处理 Klenow 片段以获得平末端。用 BamHI 消化了所得乳酸乳球菌 ilvD 编码区片段并用 QIAGEN 凝胶回收试剂盒 (QIAGEN) 进行了凝胶纯化。用 ApaLI 消化了质粒 pDM1 并用 DNA 聚合酶的 Klenow 片段处理以获得平末端,然后用 BamHI 进行了消化。上述凝胶纯化的乳酸乳球菌 ilvD 编码区片段被连接至质粒 pDM1 的 BamHI 和 ApaLI(平末端) 位点。连接混合物被转入大肠杆菌 Top10 细胞 (Invitrogen ;Carlsbad, CA)。转化体被铺板于 LB 氯霉素平板上以进行选择。通过用 SalI 消化,产生一个具有预期的 5.3kbp 大小的片段,筛选了阳性克隆。通过 DNA 测序进一步验证了阳性克隆。将正确的克隆称为 pDM1-ilvD(乳酸乳球菌 (L. lactis)),其具有从 P30 开始表达的乳酸乳球菌 (L. lactis)ilvD 编码区。

[0211] pDM1-ilvD(乳酸乳球菌 (L. lactis))-PldhL1-ilvC(荧光假单胞菌 (P. fluorescens)PF5) 的 P30 启动子被枯草芽孢杆菌 (B. subtilis)groE 启动子 (PgroE) 取代,该启动子融合到 lacO 操纵子序列和 lacI 阻遏蛋白基因上。质粒 pHTO1(Mo Bi Tec, Goettingen, Germany) 用 SacI 消化并用 Klenow 片段处理以制备平末端,用 BamHI 消化,然后凝胶纯化 1,548bp 的 lacI-PgroE/lacO 片段 (SEQ ID NO :176)。将 lacI-PgroE/lacO DNA 片段克隆进 pDM1-ilvD(乳酸乳球菌 (L. lactis))-PldhL1-ilvC(荧光假单胞菌 (P. fluorescens)PF5) 的 KpnI(用 Klenow 片段处理得到平末端) 和 BamHI 位点中以取

代 P30 启动子，生成 pDM5-ilvD（乳酸乳球菌 (L.lactis))-PldhL1-ilvC（荧光假单胞菌 (P.fluorescens)PF5)。

[0212] 为了移除 ilvD（乳酸乳球菌 (L.lactis)) 片段，pDM5-ilvD（乳酸乳球菌 (L.lactis))-PldhL1-ilvC（荧光假单胞菌 (P.fluorescens)PF5) 用 NotI 和 BamHI 消化并用 CIP 处理以制备平末端。凝胶纯化 6,207bp 的 DNA 片段并使之自身连接，所述片段包含 pDM5 主链、ldhL1 启动子 (PldhL1) 和荧光假单胞菌 (P.fluorescens)PF5ilvC 编码区，生产 pDM5-PldhL1-ilvC（荧光假单胞菌 (P.fluorescens)PF5)。

[0213] 实施例 6

[0214] pDM5-PldhL1-ilvC（变异链球菌 (S.mutans))、pDM5-PldhL1-ilvC（嗜热链球菌 (S.thermophilus)) 和 pDM5-PldhL1-ilvC（肠膜明串珠菌 (L.mesenteroides)) 载体的构建

[0215] 这个实施例的目的在于描述将酮醇酸还原异构酶（编码 SEQ ID NO:27；蛋白质 SEQ ID NO:28) 的变异链球菌 (Streptococcus mutans)UA159 ilvC 编码区、酮醇酸还原异构酶 ((编码 SEQ ID NO:55；蛋白质 SEQ ID NO:56) 的嗜热链球菌 (Streptococcus thermophilus)LMD-9ilvC 编码区和酮醇酸还原异构酶（编码 SEQ ID NO:39；蛋白质 SEQ ID NO:40) 的肠膜明串珠菌肠膜亚种 (Leuconostoc mesenteroides subsp.mesenteroides) ATCC8293ilvC 编码区克隆进表达载体。

[0216] 为了克隆在与实施例 4 和 5 中的乳酸乳球菌 (L.lactis) 和荧光假单胞菌 (P.fluorescens)PF5 ilvC 基因相同的启动子控制下的变异链球菌 (S.mutans)、嗜热链球菌 (S.thermophilus) 和肠膜明串珠菌 (L.mesenteroides)ilvC 编码区，首先构建载体 pDM5-PldhL1-MCS。ldhL1 启动子来自植物乳杆菌 (L.plantarum)PN0512 基因组 DNA，它使用包含 NotI 限制性位点的引物 AA234(SEQ ID NO:190) 以及包含多克隆位点 (MCS) 的多个限制性位点和 DrdI 限制性位点的引物 AA179(SEQ ID NO:191)，利用 Phusion High-Fidelity PCR Master Mix 进行扩增。所得 DNA 片段用 NotI 和 DrdI 消化。载体 pDM5-PldhL1-ilvC（荧光假单胞菌 (P.fluorescens)Pf5) 用 NotI 和 DrdI 消化并凝胶纯化大片段 (4712bp) 以除去 PldhL1-ilvC（荧光假单胞菌 (P.fluorescens)Pf5) 序列。凝胶纯化的片段与消化的 PldhL1-MCS PCR 连接以生成载体 pDM5-PldhL1-MCS，其具有唯一的限制性位点 KasI、PacI、AvrII、SacI 和 PmeI。通过 PCR 和测序确认载体。

[0217] 变异链球菌 (Streptococcus mutans)UA159 ilvC 编码区 (SEQ ID NO:27) 得自变异链球菌 (S.mutans)UA159 基因组 DNA，它使用包含 AvrII 限制性位点和核糖体结合序列的引物 AA235(SEQ ID NO:192) 以及包含 SacI 限制性位点的引物 AA236(SEQ ID NO:193)，利用 Phusion High-Fidelity PCR Master Mix 进行扩增。嗜热链球菌 (Streptococcus thermophilus)LMD-9(ATCC BAA-491)ilvC 编码区 (SEQ ID NO:55) 通过菌落 PCR，使用包含 AvrII 限制性位点和核糖体结合序列的引物 AA237(SEQ ID NO:194) 以及包含 SacI 限制性位点的引物 AA238(SEQ ID NO:195)，利用 Phusion High-Fidelity PCR Master Mix 进行扩增。肠膜明串珠菌肠膜亚种 (Leuconostoc mesenteroides subsp.mesenteroides)ATCC 8293 ilvC 编码区 (SEQ ID NO:39) 得自肠膜明串珠菌肠膜亚种 (L.mesenteroides subsp. mesenteroides)ATCC 8293 基因组 DNA，其使用包含 AvrII 限制性位点和核糖体结合序列的引物 AA239(SEQ ID NO:196) 以及包含 SacI 限制性位点的引物 AA240(SEQ ID NO::197)，利用 Phusion High-Fidelity PCR Master Mix 进行扩增。三个 ilvC 基因使用 AvrII 和 SacI

进行酶切，并且在用 AvrII 和 SacI 消化后分别连接到对应的 pDM5-PldhL1-MCS 限制性位点中以产生载体 pDM5-PldhL1-ilvC（变异链球菌 (S.mutans))、pDM5-PldhL1-ilvC（嗜热链球菌 (S.thermophilus)) 和 pDM5-PldhL1-ilvC（肠膜明串珠菌 (L.mesenteroides))。通过 PCR 和测序确认载体。

[0218] 实施例 7

[0219] 使用 PN0512ΔIdhDΔldhL1::ilvD(L1)suf::P5P4^{+}glgB::Tn5-PgroE-kivD(o)-sadB(o) 制备异丁醇，其包含载体 pDM5-PldhL1-ilvC（乳酸乳球菌 (L.lactis))、pDM5-PldhL1-ilvC（变异链球菌 (S.mutans))、pDM5-PldhL1-ilvC（嗜热链球菌 (S.thermophilus))、pDM5-PldhL1-ilvC（肠膜明串珠菌 (L.mesenteroides)) 或 pDM5-PldhL1-ilvC（荧光假单胞菌 (P.fluorescens)Pf5)

[0220] 这个实施例的目的在于展示与包含载体 pDM5-PldhL1-ilvC（荧光假单胞菌 (P.fluorescens)Pf5) 的 PN0512ΔIdhDΔldhL1::ilvD(L1)suf::P5P4+glgB::Tn5-PgroE-kivD(o)-sadB(o) 相比，在包含载体 pDM5-PldhL1-ilvC（乳酸乳球菌 (L.lactis))、pDM5-PldhL1-ilvC（变异链球菌 (S.mutans))、pDM5-PldhL1-ilvC（嗜热链球菌 (S.thermophilus)) 或 pDM5-PldhL1-ilvC（肠膜明串珠菌 (L.mesenteroides)) 的 PN0512ΔIdhDΔldhL1::ilvD(L1)suf::P5P4^{+}glgB::Tn5-PgroE-kivD(o)-sadB(o) 中提高的异丁醇产量。

[0221] 为了构建表达异丁醇生物合成途径的基因的重组植物乳杆菌 (Lactobacillus plantarum)，如一般方法所述制备 PN0512ΔIdhDΔldhL1::ilvD(L1)suf::P5P4^{+}glgB::Tn5-PgroE-kivD(o)-sadB(o) 的感受态细胞并用质粒 pDM5-PldhL1-ilvC（荧光假单胞菌 (P.fluorescens)Pf5)、pDM5-PldhL1-ilvC（乳酸乳球菌 (L.lactis))、pDM5-PldhL1-ilvC（变异链球菌 (S.mutans))、pDM5-PldhL1-ilvC（嗜热链球菌 (S.thermophilus)) 或 pDM5-PldhL1-ilvC（肠膜明串珠菌 (L.mesenteroides)) 进行转化，分别生成 PN0512ΔIdhDΔldhL1::ilvD(L1)suf::P5P4^{+}glgB::Tn5-PgroE-kivD(o)-sadB(o)/pDM5-PldhL1-ilvC（荧光假单胞菌 (P.fluorescens)Pf5)、PN0512ΔIdhDΔldhL1::ilvD(L1)suf::P5P4^{+}glgB::Tn5-PgroE-kivD(o)-sadB(o)/pDM5-PldhL1-ilvC（乳酸乳球菌 (L.lactis))、PN0512ΔIdhDΔldhL1::ilvD(L1)suf::P5P4^{+}glgB::Tn5-PgroE-kivD(o)-sadB(o)/pDM5-PldhL1-ilvC（变异链球菌 (S.mutans))、PN0512ΔIdhDΔldhL1::ilvD(L1)suf::P5P4^{+}glgB::Tn5-PgroE-kivD(o)-sadB(o)/pDM5-PldhL1-ilvC（嗜热链球菌 (S.thermophilus)) 和 PN0512ΔIdhDΔldhL1::ilvD(L1)suf::P5P4^{+}glgB::Tn5-PgroE-kivD(o)-sadB(o)/pDM5-PldhL1-ilvC（肠膜明串珠菌 (L.mesenteroides))。通过从内源性基因中天然表达提供异丁醇途径的第一个酶，乙酰乳酸合酶。

[0222] 将包含载体 pDM5-PldhL1-ilvC（荧光假单胞菌 (P.fluorescens)Pf5)、pDM5-PldhL1-ilvC（乳酸乳球菌 (L.lactis))、pDM5-PldhL1-ilvC（变异链球菌 (S.mutans))、pDM5-PldhL1-ilvC（嗜热链球菌 (S.thermophilus)) 或 pDM5-PldhL1-ilvC（肠膜明串珠菌 (L.mesenteroides)) 的五个 PN0512ΔIdhDΔldhL1::ilvD(L1)suf::P5P4^{+}glgB::Tn5-PgroE-kivD(o)-sadB(o) 菌株接种到在培养瓶中包含 10μg/mL 的氯霉素的乳杆菌 MRS 培养基中，并且在 30℃下有氧生

长过夜。使用过夜培养物接种到 20mL 的 MRS 培养基并生长至初始 OD600 为 0.3，所述培养基在 120mL 血清瓶中包含 100mM 的 3- 吗啉丙磺酸 (MOPS)pH7，10μg/mL 氯霉素，40μM 柠檬酸铁和 0.5mM 半胱氨酸。培养物在振荡 (100RPM) 条件下在 37℃厌氧生长 72 小时。将培养物样品在 4℃下以 3700×g 离心 10 分钟并将上清液滤过 0.2μm 的过滤器 (Pall Life Sciences，Ann Arbor，MI)。GC 分析过滤后的上清液，所述 GC 分析使用 HP-Innowax Polyethylene Glycol 柱 (19091N-113，Agilent Technologies，Santa Clara，CA) 和火焰电离检测。表 3 中的结果示出了五个菌株的异丁醇产量。包含载体 pDM5-PldhL1-ilvC(乳酸乳球菌 (L. lactis)) 的 PN0512ΔIdhDΔldhL1::ilvD(Ll)suf::P5P4^{+}glgB::Tn5-PgroE-kivD(o)-sadB(o) 生产的异丁醇量是 4.9mM，该产量大约比包含载体 pDM5-PldhL1-ilvC(荧光假单胞菌 (P. fluorescens)Pf5) 的 PN0512ΔIdhDΔldhL1::ilvD(Ll)suf::P5P4^{+}glgB::Tn5-PgroE-kivD(o)-sadB(o) 生产的异丁醇产量 (1.0mM) 高 5 倍。其它 KARI 每个具有比 Pf5 高至少 3 倍的异丁醇产量。

[0223] 表 3：重组植物乳杆菌 (Lactobacillus plantarum) 菌株的异丁醇产量。

[0224]

菌株	异丁醇（mM）
PN0512ΔIdhDΔldhL1::ilvD（Ll）suf::P5P4^{+} glgB::Tn5-PgroE-kivD（o）-sadB（o）/pDM5-PldhL1-ilvC（荧光假单胞菌（P. fluorescens）Pf5）	1.0
PN0512ΔIdhDΔldhL1::ilvD（Ll）suf::P5P4^{+} glgB::Tn5-PgroE-kivD（o）-sadB（o）/pDM5-PldhL1-ilvC（乳酸乳球菌（L. lactis））	4.9
PN0512ΔIdhDΔldhL1::ilvD（Ll）suf::P5P4^{+} glgB::Tn5-PgroE-kivD（o）-sadB（o）/pDM5-PldhL1-ilvC（变异链球菌（S. mutans））	3.8
PN0512ΔIdhDΔldhL1::ilvD（Ll）suf::P5P4^{+} glgB::Tn5-PgroE-kivD（o）-sadB（o）/pDM5-PldhL1-ilvC（嗜热链球菌（S. thermophilus））	3.2
PN0512ΔIdhDΔldhL1::ilvD（Ll）suf::P5P4^{+} glgB::Tn5-PgroE-kivD（o）-sadB（o）/pDM5-PldhL1-ilvC（肠膜明串珠菌（L. mesenteroides））	3.6

[0225] 实施例 8

[0226] 酵母中用于异丁醇生产的不同 KARI 酶的表达载体构建

[0227] 使用双质粒系统工程化酵母中的异丁醇途径。表达酮醇酸还原异构酶 (KARI) 和乙酰乳酸合酶 (ALS) 的第一个质粒称为 pYZ090(SEQ ID NO：198)。构建 pYZ090 以包含具有得自枯草芽孢杆菌 (Bacillus subtilis) 的 alsS 基因编码区的嵌合基因 (nt 位点 457-2172)，它从酵母 CUP1 启动子 (nt 2-449) 开始表达并后接 CYC1 终止子 (nt 2181-2430) 以表达 ALS，并且具有得自乳酸乳球菌 (Lactococcus lactis) 的 ilvC 基因编码区的嵌合基因 (nt 3634-4656)，它从酵母 ILV5 启动子 (2433-3626) 开始表达并后接 ILV5 终止子 (nt 4682-5304) 以表达 KARI。这个载体中的乳酸乳球菌 (L. lactis)KARI 编码区是通过 PCR 获得的 DNA 片段，所述 PCR 使用 LLKARI-PmeI(SEQ ID NO：199) 和 LLKARI-SfiI

SEQ ID NO:200) 的引物组并用 pLH475-IlvC(L1) 作为模板。为了构建 pLH475-IlvC(L1), ilvC-L1 编码区用 IlvC(Lactis)-F 和 IlvC(Lactis)-R(SEQ ID NO:201 和 202) 引物组,使用模板 pDM5-PldhL1-ilvC(乳酸乳球菌 (L.lactis)) 进行扩增,这在上述实施例 4 中进行了描述。PCR 产物用 AvrII 和 SfiI 消化并被克隆进相应的 pLH475- 基载体位点中,生成构建体 pLH475-IlvC(L1)(SEQ ID NO:203),也称为 pLH475-IlvC(乳酸乳球菌 (L.lactis))。

[0228] 质粒 pYZ091(SEQ ID NO:204) 与 pYZ090 相同,不同的是乳酸乳球菌 (L.lactis) ilvC 编码区被荧光假单胞菌 (Pseudomonas fluorescens)ilvC 编码区 (ilvC(Pf-5) 取代。IlvC(Pf-5) 编码区用 pILVCy-PmeII(SEQ ID NO:205) 和 pilvCy-SfiI(SEQ ID NO:206) 引物组,利用 pLH532 作为模板 DNA 进行扩增。pLH532(SEQ ID NO:207) 是 pHR81 载体 (ATCC #87541),其中 ILV5 编码区 (nt 8118-9167) 位于 FBA 启动子 (nt 7454-8110) 和 CYC1 终止子 (nt 9176-9425) 之间,并且得自荧光假单胞菌 (P.fluorescence)Pf-5(nt 10192-11208) 的 IlvC 编码区位于 ILV5 启动子 (nt 11200-12390) 和 ILV5 终止子 (nt 9434-10191) 之间。这个基因是 SEQ ID NO:207 中的 pLH532 序列的反向互补序列。Pf-5 编码区已经经密码子优化以在啤酒糖酵母 (S.cerevisiae) 中表达。

[0229] 质粒 pYZ058(SEQ ID NO:208) 与 pYZ090 相同,不同的是乳酸乳球菌 (L.lactis) ilvC 编码区被啤酒糖酵母 (Saccharomyces cerevisiae)ILV5 编码区 (ILV5(Sc)) 取代。啤酒糖酵母 (S.cerevisiae)ILV5 编码区无线粒体信号肽,它用 pilv5-PmeI(SEQ ID NO:209) 和 pilv5-SfiI(SEQ ID NO:210) 引物组,利用 pLH532 作为模板 DNA 进行扩增。

[0230] 具有剩余的异丁醇途径的第二质粒称为 pYZ067(SEQ ID NO:211)。构建这个载体以包含以下嵌合基因 :1) 得自变异链球菌 (S.mutans)UA159 的 ilvD 基因编码区,其具有 C- 末端 Lumio 标记 (nt 位点 2260-3996),该标记从酵母 FBA1 启动子 (nt 1161-2250) 开始表达,后接 FBA1 终止子 (nt 4005-4317),用于表达二羟酸脱水酶 (DHAD),2) 马肝 ADH(nt 4680-5807) 的编码区,它从酵母 GPM1 启动子 (nt 5819-6575) 开始表达,后接 ADH1 终止子 (nt 4356-4671),用于表达醇脱氢酶,和 3) 得自乳酸乳球菌 (Lactococcuslactis)(nt 7175-8821) 的 KivD 基因编码区,它从酵母 TDH3 启动子 (nt 8830-9493) 开始表达,后接 TDH3 终止子 (nt 6582-7161),用于表达酮异戊酸脱羧酶。

[0231] 异丁醇生产宿主菌株

[0232] 菌株 NYLA84 具有基因型 BY4700 pdc6::P_{GPM1}-sadB-ADH1t pdc1::P_{PDC1}-ilvD-FBA1tΔhis3Δhxk2 pdc5::kanMX4,描述于 2009 年 9 月 29 日提交的美国临时专利申请 61/246,709 中,其用于异丁醇生产。该菌株通过插入灭活啤酒糖酵母 (S.cerevisiae) 的内源 PDC1、PDC5 和 PDC6 基因进行构建。PDC1、PDC5 和 PDC6 基因编码三个丙酮酸脱羧酶的主要同功酶。

[0233] pdc6::P_{GPM1}-sadB 整合盒和 PDC6 删除的构建 :

[0234] 通过将来自 pRS425::GPM-sadB(如上所述) 的 GPM-sadB-ADHt 片段 (SEQ ID NO:156) 与来自 pUC19-URA3r 的 URA3r 基因相联,构建 pdc6::P_{GPM1}-sadB-ADH1t-URA3r 整合盒。pUC19-URA3r(SEQ ID NO:212) 包含来自 pRS426(ATCC #77107) 的 URA3 标记,该标记两翼为 75bp 的同源重复序列,以允许体内同源重组和 URA3 标记的移除。以 pRS425::GPM-sadB 和 pUC19-URA3r 质粒 DNA 作为模板,利用 Phusion DNA 聚合酶 (New England Biolabs Inc., Beverly, MA ;目录号 F-540S) 和引物 114117-11A 至 114117-11D(SEQ ID NO:213、214、215

和 216) 以及 114117-13A 和 114117-13B(SEQ ID NO :217 和 218),通过重叠延伸 PCR(SOE PCR)(如 Horton 等人 (1989)Gene 77 :61-68 所述) 将上述两种 DNA 片段相连。

[0235] SOE PCR 的外侧引物 (114117-13A 和 114117-13B) 包含 5′ 和 3′ ～ 50bp 分别与 PDC6 启动子和终止子的上游和下游区域同源的区域。利用标准的遗传学技术 (Methods in Yeast Genetics,2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY,第 201-202 页),将完整的盒 PCR 片段转入了 BY4700(ATCC #200866),并且将转化体在 30℃培养于不含尿嘧啶且补充了 2% 葡萄糖的合成的完全培养基上。利用引物 112590-34G 和 112590-34H(SEQ ID NO :219 和 220),以及 112590-34F 和 112590-49E(SEQ ID NO :221 和 222),通过 PCR 对转化体进行了筛选,以验证 PDC6 编码区的删除在 PDC6 基因座处的整合。通过按照标准规程在 30℃涂布于补充了 2% 葡萄糖和 5-FOA 的合成的完全培养基上,对上述 URA3r 标记进行了回收利用。通过将来自上述 5-FOA 平板的菌落接种至 SD-URA 培养基上,生长的抑制验证了标记的移除。得到的经过鉴定的菌株具有下列基因型 :BY4700 pdc6::P_{GPM1}-sadB-ADH1t。

[0236] pdc1::P_{PDC1}-ilvD 整合盒和 PDC1 删除的构建 :

[0237] 利用 SOE PCR(如 Horton 等人 (1989)Gene 77 :61-68 所述), 通过将来自 pLH468(参见上文) 的 ilvD-FBA1t 片段 (SEQ ID NO :223) 与来自 pUC19-URA3r 的 URA3r 基因相连,构建了 pdc1::P_{PDC}1-ilvD-FBA1t-URA3r 整合盒,其中所述 SOE PCR 用 pLH468 和 pUC19-URA3r 质粒 DNA 作为模板,利用 Phusion DNA 聚合酶 (New England Biolabs Inc., Beverly, MA ;目录号 F-540S) 和引物 114117-27A 至 114117-27D(SEQ ID NO :224、225、226 和 227) 进行。

[0238] SOE PCR 的外侧引物 (114117-27A 和 114117-27D) 包含 5′ 和 3′ ～ 50bp 与 PDC1 启动子的下游区域和 PDC1 编码区的下游区域同源的区域。利用标准的遗传学技术 (Methods in Yeast Genetics,2005,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,NY,第 201-202 页),将完整的盒 PCR 片段转入了 BY4700 pdc6::P_{GPM1}-sadB-ADH1t, 并将转化体在 30℃培养于不含尿嘧啶且补充了 2% 葡萄糖的合成的完全培养基上。利用引物 114117-36D 和 135(SEQ ID NO 228 和 229),以及引物 112590-49E 和 112590-30F(SEQ ID NO :222 和 230),通过 PCR 对转化体进行了筛选,以验证 PDC1 编码区的删除在 PDC1 基因座处的整合。通过按照标准规程在 30℃涂布于补充了 2% 葡萄糖和 5-FOA 的合成的完全培养基上,对上述 URA3r 标记进行了回收利用。通过将来自上述 5-FOA 平板的菌落接种至 SD-URA 培养基上,生长的抑制验证了标记的移除。得到的经过鉴定的菌株“NYLA67”具有下列基因型 :BY4700 pdc6::P_{GPM1}-sadB-ADH1t pdc1::P_{PDC1}-ilvD-FBA1t。

[0239] HIS3 删除

[0240] 为了删除内源的 HIS3 编码区,从 URA3r2 模板 DNA(SEQ ID NO :231)PCR 扩增了 his3::URA3r2 盒。URA3r2 包含来自 pRS426(ATCC #77107) 的 URA3 标记,该标记两翼为 500bp 的同源重复序列,以允许体内同源重组和 URA3 标记的移除。PCR 系利用 Phusion DNA 聚合酶和引物 114117-45A 和 114117-45B(SEQ ID NO :232 和 233) 进行,其产生了～ 2.3kb 的 PCR 产物。每种引物的 HIS3 部分来源于 HIS3 启动子上游的 5′ 区域和编码区下游的 3′ 区域,如此 URA3r2 标记的整合导致 HIS3 编码区的置换。使用标准遗传技术 (Methods in Yeast Genetics,2005,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,NY,

第 201-202 页）将 NYLA67 转化到 PCR 产物中，并且在合成的完全培养基上，在 30℃下选择转化子，所述培养基缺乏尿嘧啶并补充有 2％的葡萄糖。通过在 30℃下将转化子接种到缺乏组氨酸并补充有 2％葡萄糖的合成完全培养基上来筛选转化子以验证整合正确。通过按照标准规程在 30℃下置于补充有 2％葡萄糖和 5-FOA 的合成完全培养基上再利用 URA3r 标记。通过将来自上述 5-FOA 平板的菌落接种至 SD-URA 培养基上，生长的抑制确认了标记的移除。所得经鉴定的菌株称为 NYLA73，它具有基因型 :BY4700 pdc6::P_{GPM1}-sadB-ADH1t pdc1::P_{PDC1}-ilvD-FBA1t Δhis3。

[0241] pdc5::kanMX 整合盒以及 PDC5 删除的构建 :

[0242] 利用 Phusion DNA 聚合酶和引物 PDC5::KanMXF 和 PDC5::KanMXR(SEQ ID NO :234 和 235)，从菌株 YLR134W 染色体 DNA(ATCC No.4034091)PCR 扩增了 pdc5::kanMX4 盒，产生了～ 2.2kb 的 PCR 产物。每种引物的 PDC5 部分来源于 PDC5 启动子上游的 5′ 区域和编码区下游的 3′ 区域，如此 kanMX4 标记的整合导致 PDC5 编码区的置换。使用标准遗传技术 (Methods in Yeast Genetics,2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY,第 201-202 页）将 PCR 产物转化到 NYLA73 中，并且在补充有 1％乙醇和遗传霉素 (200μg/mL) 的 YP 培养基上，在 30℃下选择转化子。通过使用引物 PDC5kofor 和 N175(SEQ ID NO :236 和 237) 的 PCR 筛选转化子以验证在 PDC 基因座上发生的 PDC5 编码区取代整合正确。经鉴定的正确转化子具有基因型 :BY4700 pdc6::P_{GPM1}-sadB-ADH1t pdc1::P_{PDC1}-ilvD-FBA1t Δhis3 pdc5::kanMX4。该菌株称为 NYLA74。

[0243] 删除 HXK2（己糖激酶 II）:

[0244] hxk2::URA3r 盒通过 PCR 从 URA3r2 模板（如上所述）中扩增出来，所述 PCR 使用 Phusion DNA 聚合酶和引物 384 和 385(SEQ ID NO :238 和 239)，生成～ 2.3kb 的 PCR 产物。每种引物的 HXK2 部分来源于 HXK2 启动子上游的 5′ 区域和编码区下游的 3′ 区域，使得 URA3r2 标记的整合导致 HXK2 编码区的置换。使用标准遗传技术 (Methods in Yeast Genetics,2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY,第 201-202 页）将 PCR 产物转化到 NYLA73 中，并且在缺乏尿嘧啶并补充有 2％葡萄糖的合成完全培养基上，在 30℃下选择转化子。通过使用引物 N869 和 N871(SEQ ID NO :240 和 241) 的 PCR 筛选转化子以验证在 HXK2 基因座上发生的 HXK2 编码区取代整合正确。通过按照标准规程在 30℃涂布于补充了 2％葡萄糖和 5-FOA 的合成的完全培养基上，对上述 URA3r2 标记进行回收利用。通过从 5-FOA 平板上涂布接种菌落到 SD-URA 培养基上以验证生长缺失来证实标记的移除，并且通过使用引物 N946 和 N947(SEQ ID NO :242 和 243) 的 PCR 来验证正确的标记移除。所得经鉴定的菌株称为 NYLA83，它具有基因型 :BY4700 pdc6::P_{GPM1}-sadB-ADH1tpdc1::P_{PDC1}-ilvD-FBA1t Δhis3 Δhxk2。

[0245] pdc5::kanMX 整合盒以及 PDC5 删除的构建

[0246] pdc5::kanMX4 盒如上所述进行 PCR- 扩增。将 PCR 片段转化到 NYLA83 中并如上所述选择和筛选转化子。经鉴定的正确转化子称为 NYLA84，它具有基因型 :BY4700 pdc6::P_{GPM1}-sadB-ADH1t pdc1::P_{PDC1}-ilvD-FBA1t Δhis3 Δhxk2 pdc5::kanMX4。

[0247] 异丁醇生产

[0248] 使用 LiAc/PEG 方法将质粒 pYZ067 和 pYZ090、pYZ091 或 pYZ058 转化到酵母菌株 NYLA84 中，并且在酵母琼脂培养平板上选择转化子，所述平板包含酵母缺陷培养基（无组

氨酸和尿嘧啶），其补充有 2％的葡萄糖和 0.1％的乙醇 (SEG)。在 30℃生长 5-6 天后，将单菌落涂布接种到包含 0.1％乙醇和 2％葡萄糖 (SEG 平板）作为碳源的相似琼脂平板上，并且在 30℃下培养 2-3 天，随后进行摇瓶测试。

[0249] 首先将来自单菌落的菌斑接种到 3mL 的 SEG(2％葡萄糖，0.1％ EtOH) 培养基中并在 30℃下在 150rpm 的滚筒中生长过夜 (20 小时）。将过夜培养物接种到在 125mL 烧瓶内的 20mL 的 SEG 培养基中至 OD600 为约 0.3，所述烧瓶具有牢固的安全盖。培养物在 30℃下，以 150rpm 振荡生长。在不同时间点采样以按照一般方法进行 HPLC 分析。

[0250] 如图 4 和 5 图所示的结果指示包含得自乳酸乳球菌 (L. lactis) 的 IlvC 编码区的酵母菌株比包含得自荧光假单胞菌 (Pseudomonas fluorescens) 的 ilvC 和得自啤酒糖酵母 (Saccharomyces cerevisiae) 的 ILV5 的酵母菌株长得更快并且生产更多的异丁醇。

[0001]

序列表

<110> ButamaxTM Advanced Biofuels LLC
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Suh, Wonchul
Paul, Brian
Nagarajan, Vasantha
Tomb, Jean-Francois

<120> 使用高效酮醇酸还原异构酶发酵生产异丁醇

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<151> 2009-09-29

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[0003]

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[0005]

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gttgaaggtt ctgctgtacc atcactattt ggtattcaac aagacgcttc aggtcaagca 480

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[0007]

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tatgatgttg ttatcggtat ccgtccagga cgctcttttg ataaagctaa ggaaaatggt 180

ttcgatgtat atcctgtggc agaagcaacg aaacaagctg acgttatcat ggttttaatt 240

ccagatgaaa tccaaggtga tgtatataaa aatgaaattg aacctaattt agaagctggt 300

aacgcacttg catttgctca tggtttcaat attcactttg gtgttattca acccccctgca 360

gatgtcgatg ttttcttagt tgcaccaaaa ggaccaggtc acttagttag acgtacattt 420

gaagaaggta gtgcagtgcc atctttattt ggtgttcaac aagatgctac aggtcatgca 480

agagatattg cattaagtta tgcaaaaggt atcggatcta caagagctgg tgtaattgaa 540

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Gly Lys Lys Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala His
20 25 30

Ala Gln Asn Leu Lys Asp Asn Gly Tyr Asp Val Val Ile Gly Ile Arg
35 40 45

Pro Gly Arg Ser Phe Asp Lys Ala Lys Glu Asn Gly Phe Asp Val Tyr
50 55 60

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

Pro Asp Glu Ile Gln Gly Asp Val Tyr Lys Asn Glu Ile Glu Pro Asn
85 90 95

Leu Glu Ala Gly Asn Ala Leu Ala Phe Ala His Gly Phe Asn Ile His
100 105 110

Phe Gly Val Ile Gln Pro Pro Ala Asp Val Asp Val Phe Leu Val Ala
115 120 125

Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Glu Glu Gly Ser
130 135 140

Ala Val Pro Ser Leu Phe Gly Val Gln Gln Asp Ala Thr Gly His Ala
145 150 155 160

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

Gly Val Ile Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Ile His Lys Leu Ile Gln Ser
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Gln Pro Glu Leu Ala Tyr
210 215 220

Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr Glu
225 230 235 240

Gly Gly Met Asp Asn Val Arg Tyr Ser Ile Ser Asn Thr Ala Glu Tyr
245 250 255

[0009]

Gly Asp Tyr Val Ser Gly Arg Arg Ile Ile Thr Pro Asp Val Lys Asp
260 265 270

Asn Met Lys Ala Val Leu Thr Asp Ile Gln Asn Gly Lys Phe Ala Asn
275 280 285

Ser Phe Val Glu Asp Asn Lys Asn Gly Phe Lys Glu Phe Tyr Lys Met
290 295 300

Arg Glu Glu Gln Ala Gly His Pro Ile Glu Lys Val Gly Arg Lys Leu
305 310 315 320

Arg Asp Met Met Pro Phe Ile Lys Ser Lys Ser Ile Glu Lys
325 330

<210> 9
<211> 1002
<212> DNA
<213> 表皮葡萄球菌 (Staphylococcus epidermidis)

<400> 9
atgacaaaag tatattacga tgaaacagta actcaagatg cattacaagg taaaaaaatt 60

gctgtcattg gttatggctc acaaggacat gcacatgcac aaaatttaaa ggacaatggt 120

tatgatgtag tcattggtct acgtccagga cgatcattta ataaagctaa agaagatgga 180

tttgatgttt atacggtaag tgatgctaca caacaagctg atgtagtgat ggtactattg 240

cctgatgaaa ttcaaggtaa agtatataac gaggaaatta aaccacattt agaaaaagga 300

aatgctttag cattcgcaca cggttttaat atccacttca gtgttatcga accacctagt 360

gatgtcgatg tctttttagt agcacctaaa ggaccaggtc atttagttag acgtacattt 420

gttgaaggaa gtgccgtacc agcattattt ggtgttcaac aagatgctac aggccaagca 480

agaaacattg ctttaagcta cgcaaaaggc attggtgcta ctcgtgccgg ggtcattgaa 540

acgacattta aagaagaaac tgaaacagat ttattcggtg aacaagctgt tctttgtgga 600

ggagtttcca aattaattca gagtggtttc gaaacacttg tagaagcagg ttaccaacct 660

gaattagctt attttgaagt cttacacgaa atgaaattaa ttgttgattt aatgtatgaa 720

ggcggaatgg aaaacgttcg ttattctatc tctaacactg ctgaatttgg cgactatgtt 780

tctggaccaa gagtaattac acctgatgtt aaagaaaata tgaaaaaagt acttgaagat 840

attcaaaatg gtaactttag ccgtagattt gttgaagata acaaaaatgg ctttaaagaa 900

ttctatcaat tacgtgaaga tcaacatggt catcaaattg aacaagttgg acgtgaatta 960

agagaaatga tgccattcat taaatctaaa agtattgaaa aa 1002

<210> 10
<211> 334
<212> PRT
<213> 表皮葡萄球菌 (Staphylococcus epidermidis)

[0010]

<400> 10

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Met Thr Lys Val Tyr Tyr Asp Glu Thr Val Thr Gln Asp Ala Leu Gln
1               5                  10                  15

Gly Lys Lys Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala His
            20                  25                  30

Ala Gln Asn Leu Lys Asp Asn Gly Tyr Asp Val Val Ile Gly Leu Arg
        35                  40                  45

Pro Gly Arg Ser Phe Asn Lys Ala Lys Glu Asp Gly Phe Asp Val Tyr
    50                  55                  60

Thr Val Ser Asp Ala Thr Gln Gln Ala Asp Val Val Met Val Leu Leu
65                  70                  75                  80

Pro Asp Glu Ile Gln Gly Lys Val Tyr Asn Glu Glu Ile Lys Pro His
                85                  90                  95

Leu Glu Lys Gly Asn Ala Leu Ala Phe Ala His Gly Phe Asn Ile His
            100                 105                 110

Phe Ser Val Ile Glu Pro Pro Ser Asp Val Asp Val Phe Leu Val Ala
        115                 120                 125

Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Val Glu Gly Ser
    130                 135                 140

Ala Val Pro Ala Leu Phe Gly Val Gln Gln Asp Ala Thr Gly Gln Ala
145                 150                 155                 160

Arg Asn Ile Ala Leu Ser Tyr Ala Lys Gly Ile Gly Ala Thr Arg Ala
                165                 170                 175

Gly Val Ile Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe
            180                 185                 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Val Ser Lys Leu Ile Gln Ser
        195                 200                 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Gln Pro Glu Leu Ala Tyr
    210                 215                 220

Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr Glu
225                 230                 235                 240

Gly Gly Met Glu Asn Val Arg Tyr Ser Ile Ser Asn Thr Ala Glu Phe
                245                 250                 255
```

[0011]

Gly Asp Tyr Val Ser Gly Pro Arg Val Ile Thr Pro Asp Val Lys Glu
260 265 270

Asn Met Lys Lys Val Leu Glu Asp Ile Gln Asn Gly Asn Phe Ser Arg
275 280 285

Arg Phe Val Glu Asp Asn Lys Asn Gly Phe Lys Glu Phe Tyr Gln Leu
290 295 300

Arg Glu Asp Gln His Gly His Gln Ile Glu Gln Val Gly Arg Glu Leu
305 310 315 320

Arg Glu Met Met Pro Phe Ile Lys Ser Lys Ser Ile Glu Lys
325 330

<210> 11
<211> 1002
<212> DNA
<213> 生性葡萄球菌 (Staphylococcus saprophyticus)

<400> 11
atgacaacag tttattatga ccaatcagtt acgaaagacg ctttacaagg taaaaaagta 60

gcaatcatag gctatggttc tcaaggacac gcacatgctc aaaatttaaa agacaacggt 120

tatgatgtca ttatcggaat cagacctgga tgttcttttg acaaagctaa agatgatggc 180

ttcgaagtat atcctgtaga tgaagcagca aaacaagctg atgtcatcat ggtgttatta 240

ccagatgaaa ttcaaggcca agtatataaa gaagaaattg aaccaaactt agaagcaaac 300

aatgcattag tattcgcgca tggttttaat attcatttcg gtgttattca accaccagaa 360

aacgtagatg tattcttagt agcgcctaaa ggacctggac atttagtacg tcgtacattt 420

gctgaaggaa gcgcagtccc tgccctattc gcagttgaac aagatccgag tggtgaagct 480

agagatttag cattaagcta tgctaaaggt atcggtgcaa cacgtgcagg tgtattagaa 540

acatcattta aagaagaaac agaaacagat ttattcggtg aacaagcagt gctttgtggt 600

ggtacgacta aattagtaca atctggtttc gaaacgttag tagaagcagg ttaccaacca 660

gaaattgcat actttgaagt attgcatgaa atgaaattga ttgttgattt aatgtatgaa 720

ggcggtatgg aaaatatgcg ctattcaatt tcaaatacag ctgaatttgg tgactatgtt 780

tctggaccac gtattatcac accggatgtt aaagataata tgaaagctgt attagatgat 840

attcaaaaag gaaacttcag tgatcgattc attaaagata atcaaaataa ttttgaagaa 900

ttccataaat taagagaaga acaacatggt catcaaatcg aagcggttgg tagagaactc 960

cgtgatatga tgccattcat caaatctaag agcattgaaa aa 1002

<210> 12
<211> 334
<212> PRT
<213> 生性葡萄球菌 (Staphylococcus saprophyticus)

[0012]

<400> 12

Met Thr Thr Val Tyr Tyr Asp Gln Ser Val Thr Lys Asp Ala Leu Gln
1 5 10 15

Gly Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala His
20 25 30

Ala Gln Asn Leu Lys Asp Asn Gly Tyr Asp Val Ile Ile Gly Ile Arg
35 40 45

Pro Gly Cys Ser Phe Asp Lys Ala Lys Asp Asp Gly Phe Glu Val Tyr
50 55 60

Pro Val Asp Glu Ala Ala Lys Gln Ala Asp Val Ile Met Val Leu Leu
65 70 75 80

Pro Asp Glu Ile Gln Gly Gln Val Tyr Lys Glu Glu Ile Glu Pro Asn
85 90 95

Leu Glu Ala Asn Asn Ala Leu Val Phe Ala His Gly Phe Asn Ile His
100 105 110

Phe Gly Val Ile Gln Pro Pro Glu Asn Val Asp Val Phe Leu Val Ala
115 120 125

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

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

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

Gly Val Leu Glu Thr Ser Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Thr Lys Leu Val Gln Ser
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Gln Pro Glu Ile Ala Tyr
210 215 220

Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr Glu
225 230 235 240

Gly Gly Met Glu Asn Met Arg Tyr Ser Ile Ser Asn Thr Ala Glu Phe
245 250 255

[0013]

Gly Asp Tyr Val Ser Gly Pro Arg Ile Ile Thr Pro Asp Val Lys Asp
260 265 270

Asn Met Lys Ala Val Leu Asp Asp Ile Gln Lys Gly Asn Phe Ser Asp
275 280 285

Arg Phe Ile Lys Asp Asn Gln Asn Asn Phe Glu Glu Phe His Lys Leu
290 295 300

Arg Glu Glu Gln His Gly His Gln Ile Glu Ala Val Gly Arg Glu Leu
305 310 315 320

Arg Asp Met Met Pro Phe Ile Lys Ser Lys Ser Ile Glu Lys
325 330

<210> 13
<211> 1002
<212> DNA
<213> 肉葡萄球菌 (Staphylococcus carnosus)

<400> 13
atgacaacag tttattatga cgaaacagta aaagaagatg ctttaaaagg taaaaaaatt 60

gcagtagttg gttatggttc tcaaggccat gctcatgcac aaaacttaaa agacaatggt 120

tatgacgttg taatcggtat ccgcccaggt aattctttcg ataaagctaa aaaagacggc 180

tttgatgtat atccagttga tgaagcagtg aaacaagctg atgtagtaat ggtattatta 240

cctgatgaaa tccaaggtaa agtatatgaa gaagaaatcg caccaaactt agaagctggc 300

aacgctttag cttttgcaca tggcttcaac atccactttg atgttatcca accacctgaa 360

gatgttgatg tattcttagt tgcccctaaa ggaccaggac atttagtaag acgtacattt 420

actgaaggaa gctcagtacc tgccctattc ggtgtagaac aagatgcttc tggcaatgct 480

ttcaacttag ctttaagtta tgcaaaaggt atcggagcaa caaaagctgg ggttatcgaa 540

actacattta aagaagaaac tgaaactgat ttatttggtg aacaagctgt actttgcggc 600

ggtgtaacaa gattaatcca aagtggattt gaaacattag ttgaagctgg ttaccaacca 660

gaattagctt attttgaagt attacatgaa atgaaattaa tcgttgattt aatgtatgaa 720

ggcggaatgg aaacaatgcg ttattctatc tcaaatacag cagaatacgg cgattatgtt 780

tctggtccac gtgtcatcac tgatgatgtt aaacaaaaca tgaaagctgt cttagaagat 840

atccaaaaacg gtaaattcgc aaacagtttc attgaagata ataaaaatgg tttcaaagag 900

ttctacaaat tgcgtgaaga agcacaagat catcaaattc aagaagttgg taaagaactt 960

cgtgaaatga tgccttttgt tgaaaataaa actattgaaa aa 1002

<210> 14
<211> 334
<212> PRT
<213> 肉葡萄球菌 (Staphylococcus carnosus)

[0014]

<400> 14

Met Thr Thr Val Tyr Tyr Asp Glu Thr Val Lys Glu Asp Ala Leu Lys
1 5 10 15

Gly Lys Lys Ile Ala Val Val Gly Tyr Gly Ser Gln Gly His Ala His
20 25 30

Ala Gln Asn Leu Lys Asp Asn Gly Tyr Asp Val Val Ile Gly Ile Arg
35 40 45

Pro Gly Asn Ser Phe Asp Lys Ala Lys Lys Asp Gly Phe Asp Val Tyr
50 55 60

Pro Val Asp Glu Ala Val Lys Gln Ala Asp Val Val Met Val Leu Leu
65 70 75 80

Pro Asp Glu Ile Gln Gly Lys Val Tyr Glu Glu Glu Ile Ala Pro Asn
85 90 95

Leu Glu Ala Gly Asn Ala Leu Ala Phe Ala His Gly Phe Asn Ile His
100 105 110

Phe Asp Val Ile Gln Pro Pro Glu Asp Val Asp Val Phe Leu Val Ala
115 120 125

Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Thr Glu Gly Ser
130 135 140

Ser Val Pro Ala Leu Phe Gly Val Glu Gln Asp Ala Ser Gly Asn Ala
145 150 155 160

Phe Asn Leu Ala Leu Ser Tyr Ala Lys Gly Ile Gly Ala Thr Lys Ala
165 170 175

Gly Val Ile Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Val Thr Arg Leu Ile Gln Ser
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Gln Pro Glu Leu Ala Tyr
210 215 220

Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr Glu
225 230 235 240

Gly Gly Met Glu Thr Met Arg Tyr Ser Ile Ser Asn Thr Ala Glu Tyr
245 250 255

[0015]

Gly Asp Tyr Val Ser Gly Pro Arg Val Ile Thr Asp Asp Val Lys Gln
260 265 270

Asn Met Lys Ala Val Leu Glu Asp Ile Gln Asn Gly Lys Phe Ala Asn
275 280 285

Ser Phe Ile Glu Asp Asn Lys Asn Gly Phe Lys Glu Phe Tyr Lys Leu
290 295 300

Arg Glu Glu Ala Gln Asp His Gln Ile Gln Glu Val Gly Lys Glu Leu
305 310 315 320

Arg Glu Met Met Pro Phe Val Glu Asn Lys Thr Ile Glu Lys
325 330

<210> 15
<211> 993
<212> DNA
<213> 单核细胞增多性李斯特菌（Listeria monocytogenes）

<400> 15
atgacaaaag tttattatga agatgcagta aaaaacaacg cactagaagg taaaacagta 60

gcagtaatcg ggtacggttc gcaaggtcac gcacattctc aaaatctacg tgacaatggc 120

aataacgtta ttatcggcat tcgcgaagga aaatctgccg aatctgctag aaacgatggc 180

tttgatgttt attctgttag cgaagccgct gataaagctg atgtcatcat gattcttttg 240

ccagatgaaa cacaaggcga aacatacgaa aatgaaatta aacctaacct aaaagctggt 300

aattcacttg ttttcgctca cggttttaac attcattttg acgtaattaa tcctccaagc 360

gatgtagatg tttttctagt agctccaaaa ggtcctggtc acttagttcg ccgcacattt 420

gttgaaggtg gcgctgttcc ttccctattc gctatctatc aagatgccac tggaaacgca 480

cgcgacacag ccctttccta tgcaaaaggt attggcgcaa ctcgtgctgg cgttatcgaa 540

accactttca aagaagaaac cgaaaccgat ctatttggcg aacaagcagt tctttgtggg 600

ggtgcgactc accttatcca agctggtttc gaaacacttg tagaggctgg ctaccaacca 660

gaacttgctt attttgaagt attacatgaa atgaaactaa ttgttgattt gatgtatgaa 720

ggtggtatgg aaaaaatgcg ccactcgatc tccaatacag cagaatatgg tgattatgtt 780

tccggtcctc gagttgttac agccgatacg aaaaaagcaa tgaaagaagt acttaccgac 840

attcaaaatg gtaactttgc taaatctttc atcaatgaca acaaaaatgg ttttaaagaa 900

ttccatagaa tgcgtaaaga acaacaaggt catcaaatcg aaaaagttgg tgcagaactt 960

cgcgaaatga tgccatttgt caaaccacaa cat 993

<210> 16
<211> 331
<212> PRT
<213> 单核细胞增多性李斯特菌（Listeria monocytogenes）

[0016]

<400> 16

Met Thr Lys Val Tyr Tyr Glu Asp Ala Val Lys Asn Asn Ala Leu Glu
1 5 10 15

Gly Lys Thr Val Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala His
20 25 30

Ser Gln Asn Leu Arg Asp Asn Gly Asn Asn Val Ile Ile Gly Ile Arg
35 40 45

Glu Gly Lys Ser Ala Glu Ser Ala Arg Asn Asp Gly Phe Asp Val Tyr
50 55 60

Ser Val Ser Glu Ala Ala Asp Lys Ala Asp Val Ile Met Ile Leu Leu
65 70 75 80

Pro Asp Glu Thr Gln Gly Glu Thr Tyr Glu Asn Glu Ile Lys Pro Asn
85 90 95

Leu Lys Ala Gly Asn Ser Leu Val Phe Ala His Gly Phe Asn Ile His
100 105 110

Phe Asp Val Ile Asn Pro Pro Ser Asp Val Asp Val Phe Leu Val Ala
115 120 125

Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Val Glu Gly Gly
130 135 140

Ala Val Pro Ser Leu Phe Ala Ile Tyr Gln Asp Ala Thr Gly Asn Ala
145 150 155 160

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

Gly Val Ile Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Ala Thr His Leu Ile Gln Ala
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Gln Pro Glu Leu Ala Tyr
210 215 220

Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr Glu
225 230 235 240

Gly Gly Met Glu Lys Met Arg His Ser Ile Ser Asn Thr Ala Glu Tyr
245 250 255

[0017]

Gly Asp Tyr Val Ser Gly Pro Arg Val Val Thr Ala Asp Thr Lys Lys
260 265 270

Ala Met Lys Glu Val Leu Thr Asp Ile Gln Asn Gly Asn Phe Ala Lys
275 280 285

Ser Phe Ile Asn Asp Asn Lys Asn Gly Phe Lys Glu Phe His Arg Met
290 295 300

Arg Lys Glu Gln Gln Gly His Gln Ile Glu Lys Val Gly Ala Glu Leu
305 310 315 320

Arg Glu Met Met Pro Phe Val Lys Pro Gln His
325 330

<210> 17
<211> 993
<212> DNA
<213> 格氏李斯特菌 (Listeria grayi)

<400> 17
atgacaaaag tatattatga agatgctgta aaagaaaatg cactaacggg gaagaaagtt 60

gccgttatcg gttatggatc acaaggacat gcgcattcgc aaaatctgcg tgacaatggc 120

aatgacgtta tcatcggtat ccgcgaagga cgttctaaag aaagtgcgaa aaacgacggc 180

ttcgatgttt actccgttgc cgaagcgagt aaacaagcag atgtcatcat gatccttcta 240

ccagacgaaa tccaaggaga tacatacgaa aaagaaatca gcccaaactt ggaagcagga 300

aatgccattg ttttcgccca tggtttcaac atccattttg atgtgatcaa acctcctaca 360

gatgtagatg ttttcctagt tgcaccaaaa ggccccggac atctagtaag acgtaccttt 420

gtagaaggcg gtgccgtccc ttccctcttc gccgtccacc aagatgccac tggcaaagcg 480

caggacctcg ccctttctta tgccaaagga atcggagcta cacgtgccgg cgttatcgaa 540

acaactttca aagaagaaac agaaacggat ctattcggtg aacaagctgt cctatgcggc 600

ggtgccactc accttatcca agcaggcttt gaaacacttg tagaagcagg ctatcaaccg 660

gaactcgctt acttcgaagt actgcatgag atgaaactga tcgtagacct gatgtacgaa 720

ggcggaatgg aaaaaatgcg ctattcgatc tccaatacag cagaatatgg cgactatgta 780

tccggtccgc gcatcgtgac cagcgatacg aagaaagcga tgaaagaagt attgaccgat 840

atccaaaatg gtaattttgc caaatcgttt atcgatgaca ataaaaacgg cttcaaagaa 900

tttcaccgaa tgcgcaaaga acagcaaggt catcaaatcg aagcagtcgg caaaaatctc 960

cgcgaaatga tgccgttcgt aaatccaaga aaa 993

<210> 18
<211> 331
<212> PRT
<213> 格氏李斯特菌 (Listeria grayi)

[0018]

<400> 18

Met Thr Lys Val Tyr Tyr Glu Asp Ala Val Lys Glu Asn Ala Leu Thr
1 5 10 15

Gly Lys Lys Val Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala His
20 25 30

Ser Gln Asn Leu Arg Asp Asn Gly Asn Asp Val Ile Ile Gly Ile Arg
35 40 45

Glu Gly Arg Ser Lys Glu Ser Ala Lys Asn Asp Gly Phe Asp Val Tyr
50 55 60

Ser Val Ala Glu Ala Ser Lys Gln Ala Asp Val Ile Met Ile Leu Leu
65 70 75 80

Pro Asp Glu Ile Gln Gly Asp Thr Tyr Glu Lys Glu Ile Ser Pro Asn
85 90 95

Leu Glu Ala Gly Asn Ala Ile Val Phe Ala His Gly Phe Asn Ile His
100 105 110

Phe Asp Val Ile Lys Pro Pro Thr Asp Val Asp Val Phe Leu Val Ala
115 120 125

Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Val Glu Gly Gly
130 135 140

Ala Val Pro Ser Leu Phe Ala Val His Gln Asp Ala Thr Gly Lys Ala
145 150 155 160

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

Gly Val Ile Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Ala Thr His Leu Ile Gln Ala
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Gln Pro Glu Leu Ala Tyr
210 215 220

Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr Glu
225 230 235 240

Gly Gly Met Glu Lys Met Arg Tyr Ser Ile Ser Asn Thr Ala Glu Tyr
245 250 255

[0019]

Gly Asp Tyr Val Ser Gly Pro Arg Ile Val Thr Ser Asp Thr Lys Lys
260 265 270

Ala Met Lys Glu Val Leu Thr Asp Ile Gln Asn Gly Asn Phe Ala Lys
275 280 285

Ser Phe Ile Asp Asp Asn Lys Asn Gly Phe Lys Glu Phe His Arg Met
290 295 300

Arg Lys Glu Gln Gln Gly His Gln Ile Glu Ala Val Gly Lys Asn Leu
305 310 315 320

Arg Glu Met Met Pro Phe Val Asn Pro Arg Lys
325 330

<210> 19
<211> 987
<212> DNA
<213> 铅黄肠球菌（Enterococcus casseliflavus）

<400> 19
atggtaaaag tatattacaa cgaggaagtt acagataatt taggagacaa gaagattgcg 60

atcgtcggct acggctctca agggcacgct catgcgcaaa atttacggga cacaggtcat 120

gaagtcttta tcggcattcg tgaaggaaaa tcagcggaag ccgcaagaaa cgatggattt 180

gaggttttcc cagtagctga agccgttcaa aaagcggatg tagtcatgat cttggcaccc 240

gatgagatcc aaggatcgtt gtataaaaat gagatcgaac cgaatctttc tgcgggcaat 300

gcattggcat ttgctcatgg tttcaatatt cattttgatg tgatcgcgcc accaaaagag 360

gtggatgttt ttctagttgc gccaaaagga ccaggtcatt tggtgcgtcg aacctttacc 420

gaagggtttg cggtaccagc cttgtttgca gtttatcaag atgcgactgg taacgcacaa 480

gcaacggctt tatcctatgc aaaaggcatt ggtgccacac gggtcggcgt tttggaaacg 540

acctttaaag aagagaccga aaccgatctt ttcggtgagc aagccgtact ttgtggcggt 600

ttgaccagta tgatcgaagc gggctttgaa acattggtag aagcggggta ccaaccggaa 660

ttggcttatt ttgaagtttg tcatgaattg aaactgatcg ttgatttgat ttatgaagga 720

ggcttcaaaa aaatgcgcca ttcgatctcg aatacggctg agtatggtga ctacgtttct 780

ggtgctcggg tgatcactgc acaagccaaa gaaaacatga aagccgtatt gacagacatc 840

caaaacggcc aatttgcgaa aggattcatt gaagacaatc aaaacggctt ccctgaattc 900

cataaaatgc gtgaggaaaa cggcaatcat caaattgaaa aagtcggcag cgagttacgg 960

aaaatgatgc cgtttgttgc gaaagat 987

<210> 20
<211> 329
<212> PRT
<213> 铅黄肠球菌（Enterococcus casseliflavus）

[0020]

<400> 20

Met Val Lys Val Tyr Tyr Asn Glu Glu Val Thr Asp Asn Leu Gly Asp
1 5 10 15

Lys Lys Ile Ala Ile Val Gly Tyr Gly Ser Gln Gly His Ala His Ala
20 25 30

Gln Asn Leu Arg Asp Thr Gly His Glu Val Phe Ile Gly Ile Arg Glu
35 40 45

Gly Lys Ser Ala Glu Ala Ala Arg Asn Asp Gly Phe Glu Val Phe Pro
50 55 60

Val Ala Glu Ala Val Gln Lys Ala Asp Val Val Met Ile Leu Ala Pro
65 70 75 80

Asp Glu Ile Gln Gly Ser Leu Tyr Lys Asn Glu Ile Glu Pro Asn Leu
85 90 95

Ser Ala Gly Asn Ala Leu Ala Phe Ala His Gly Phe Asn Ile His Phe
100 105 110

Asp Val Ile Ala Pro Pro Lys Glu Val Asp Val Phe Leu Val Ala Pro
115 120 125

Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Thr Glu Gly Phe Ala
130 135 140

Val Pro Ala Leu Phe Ala Val Tyr Gln Asp Ala Thr Gly Asn Ala Gln
145 150 155 160

Ala Thr Ala Leu Ser Tyr Ala Lys Gly Ile Gly Ala Thr Arg Val Gly
165 170 175

Val Leu Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe Gly
180 185 190

Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ser Met Ile Glu Ala Gly
195 200 205

Phe Glu Thr Leu Val Glu Ala Gly Tyr Gln Pro Glu Leu Ala Tyr Phe
210 215 220

Glu Val Cys His Glu Leu Lys Leu Ile Val Asp Leu Ile Tyr Glu Gly
225 230 235 240

Gly Phe Lys Lys Met Arg His Ser Ile Ser Asn Thr Ala Glu Tyr Gly
245 250 255

[0021]

Asp Tyr Val Ser Gly Ala Arg Val Ile Thr Ala Gln Ala Lys Glu Asn
260 265 270

Met Lys Ala Val Leu Thr Asp Ile Gln Asn Gly Gln Phe Ala Lys Gly
275 280 285

Phe Ile Glu Asp Asn Gln Asn Gly Phe Pro Glu Phe His Lys Met Arg
290 295 300

Glu Glu Asn Gly Asn His Gln Ile Glu Lys Val Gly Ser Glu Leu Arg
305 310 315 320

Lys Met Met Pro Phe Val Ala Lys Asp
325

<210> 21
<211> 984
<212> DNA
<213> 鹑鸡肠球菌 (Enterococcus gallinarum)

<400> 21
atggtaaaag tatattataa cgaagtgaca gacaatttag gagataagac tattgcaatc 60

attggttacg gttctcaagg acatgcccat gcacagaatt tacgggatac aggacacaac 120

gtcgtgatcg ggattcgtgc aggaaaatca gctgagaacg caaagaatga tggatttacc 180

gtgcttccag tttctgaagc tgtacaacaa gcaaatgttg tgatgatctt agctcctgat 240

gagatccaag gagaacttta cgagaaggaa attgcgccta atctttctgc aggggatgca 300

ttagcctttg ctcatggctt taatattcat tttgaggtca tcacgcctcc aaaagaggtg 360

gatgtcttcc tggtggcacc taaaggacca ggccatttgg tgcgccggac ctttagcgaa 420

ggatttgctg tccctgcgtt gtttgctgtt tatcaagatg ctactggcaa tgcacaagag 480

acagccttgt cctatgccaa aggaatcggg gctacaagag tcggtgtact agaaacaacg 540

ttcaaggaag agacggaaac agacttgttt ggggaacaag ccgtattatg tggcggactg 600

acaagtatga tcgagactgg gtttgaaacg ctggtagaag ccggctatca gccagaacta 660

gcctattttg aagtttgtca cgaaatgaaa ctgattgttg acttgattta tgaaggagga 720

ttcgccaaga tgcgtgattc aatctccaat acagcagaat atggggatta tgtctctggt 780

tcacgaatca ttaccgaaca aacaaaagcc aatatgaaga atgtcttgaa agatattcaa 840

aacggcacgt ttgccaaagg ttttattgcg gataatcaag ctggttttcc ggaattcaaa 900

aaaatgcggg aacaaaacgg gaatcatcca atcgaaaaag ttgggtcaga attaagaaaa 960

atgatgcctt ttgttacgaa agat 984

<210> 22
<211> 328
<212> PRT
<213> 鹑鸡肠球菌 (Enterococcus gallinarum)

[0022]

<400> 22

Met Val Lys Val Tyr Tyr Asn Glu Val Thr Asp Asn Leu Gly Asp Lys
1 5 10 15

Thr Ile Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala His Ala Gln
20 25 30

Asn Leu Arg Asp Thr Gly His Asn Val Val Ile Gly Ile Arg Ala Gly
35 40 45

Lys Ser Ala Glu Asn Ala Lys Asn Asp Gly Phe Thr Val Leu Pro Val
50 55 60

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

Glu Ile Gln Gly Glu Leu Tyr Glu Lys Glu Ile Ala Pro Asn Leu Ser
85 90 95

Ala Gly Asp Ala Leu Ala Phe Ala His Gly Phe Asn Ile His Phe Glu
100 105 110

Val Ile Thr Pro Pro Lys Glu Val Asp Val Phe Leu Val Ala Pro Lys
115 120 125

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

Pro Ala Leu Phe Ala Val Tyr Gln Asp Ala Thr Gly Asn Ala Gln Glu
145 150 155 160

Thr Ala Leu Ser Tyr Ala Lys Gly Ile Gly Ala Thr Arg Val Gly Val
165 170 175

Leu Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe Gly Glu
180 185 190

Gln Ala Val Leu Cys Gly Gly Leu Thr Ser Met Ile Glu Thr Gly Phe
195 200 205

Glu Thr Leu Val Glu Ala Gly Tyr Gln Pro Glu Leu Ala Tyr Phe Glu
210 215 220

Val Cys His Glu Met Lys Leu Ile Val Asp Leu Ile Tyr Glu Gly Gly
225 230 235 240

Phe Ala Lys Met Arg Asp Ser Ile Ser Asn Thr Ala Glu Tyr Gly Asp
245 250 255

[0023]

Tyr Val Ser Gly Ser Arg Ile Ile Thr Glu Gln Thr Lys Ala Asn Met
260 265 270

Lys Asn Val Leu Lys Asp Ile Gln Asn Gly Thr Phe Ala Lys Gly Phe
275 280 285

Ile Ala Asp Asn Gln Ala Gly Phe Pro Glu Phe Lys Lys Met Arg Glu
290 295 300

Gln Asn Gly Asn His Pro Ile Glu Lys Val Gly Ser Glu Leu Arg Lys
305 310 315 320

Met Met Pro Phe Val Thr Lys Asp
325

<210> 23
<211> 990
<212> DNA
<213> 溶酪巨型球菌（Macrococcus caseolyticus）

<400> 23
atggctaaag tatattatga taacgatgta aataaggaat acttaaaaaa taagaaaatt 60

gcagtattag ggtacggctc tcaaggacac gcgcatgcat tgaatcttaa ggacagtggc 120

tatgacgtag tcatcggagt acgtgaaggg cagtccagaa caaaggcaga agaagatgga 180

tttcaagcat atgacgtcag tgaagctgtg gaacgtgcgg atgttaccgt cgtccttatg 240

ccggacgaag tacagcagcg tgtgttcaac gaggaaatcg tacctcatct caaaccacaa 300

tcggctctag ttttcgctca tggatttaat gtgcatttcg gtgcaataaa agctcctgac 360

gatgttgatg tcttcttagt cgcaccaaaa ggaccaggac atcttgttcg acgcgaatat 420

gttaagggca gtgctgtccc tgcgttattt gcagtcgatc aggatgtaac tggtgatgca 480

aaggcccttg cactgaacta tgcacacggc atcggtgcaa cgcgcgccgg cgtcatcgaa 540

acgaccttca aggaagaaac agagacagac ctttttggtg agcaagcggt actctgcggc 600

ggtatcacaa agctcatcca ctacggtttt gaaacactga cggaagcagg ttatcagcca 660

gaactcgcct atttcgaagt actgcatgaa atgaagctga ttgtggacct tatgtacgag 720

ggcggtatgg agaagatgag acattccatc tcaaataccg ctgagtttgg agattatgtc 780

tctggtgcgc gcgttattac ccccgaagtt aaggaaaata tgaaacaggt attagctgat 840

atccagtctg gcacattcag caggcagttt atcgaagaca ataataatgg atttgagtct 900

tttaaatcta tgagaagtac ccaggcagga catcctatag agaaagtcgg tgcagaccta 960

agaatgatga tgccgtttat caataataac 990

<210> 24
<211> 330
<212> PRT
<213> 溶酪巨型球菌（Macrococcus caseolyticus）

[0024]

<400> 24

Met Ala Lys Val Tyr Tyr Asp Asn Asp Val Asn Lys Glu Tyr Leu Lys
1 5 10 15

Asn Lys Lys Ile Ala Val Leu Gly Tyr Gly Ser Gln Gly His Ala His
20 25 30

Ala Leu Asn Leu Lys Asp Ser Gly Tyr Asp Val Val Ile Gly Val Arg
35 40 45

Glu Gly Gln Ser Arg Thr Lys Ala Glu Glu Asp Gly Phe Gln Ala Tyr
50 55 60

Asp Val Ser Glu Ala Val Glu Arg Ala Asp Val Thr Val Val Leu Met
65 70 75 80

Pro Asp Glu Val Gln Gln Arg Val Phe Asn Glu Glu Ile Val Pro His
85 90 95

Leu Lys Pro Gln Ser Ala Leu Val Phe Ala His Gly Phe Asn Val His
100 105 110

Phe Gly Ala Ile Lys Ala Pro Asp Asp Val Asp Val Phe Leu Val Ala
115 120 125

Pro Lys Gly Pro Gly His Leu Val Arg Arg Glu Tyr Val Lys Gly Ser
130 135 140

Ala Val Pro Ala Leu Phe Ala Val Asp Gln Asp Val Thr Gly Asp Ala
145 150 155 160

Lys Ala Leu Ala Leu Asn Tyr Ala His Gly Ile Gly Ala Thr Arg Ala
165 170 175

Gly Val Ile Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Ile Thr Lys Leu Ile His Tyr
195 200 205

Gly Phe Glu Thr Leu Thr Glu Ala Gly Tyr Gln Pro Glu Leu Ala Tyr
210 215 220

Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr Glu
225 230 235 240

Gly Gly Met Glu Lys Met Arg His Ser Ile Ser Asn Thr Ala Glu Phe
245 250 255

[0025]

Gly Asp Tyr Val Ser Gly Ala Arg Val Ile Thr Pro Glu Val Lys Glu
260 265 270

Asn Met Lys Gln Val Leu Ala Asp Ile Gln Ser Gly Thr Phe Ser Arg
275 280 285

Gln Phe Ile Glu Asp Asn Asn Asn Gly Phe Glu Ser Phe Lys Ser Met
290 295 300

Arg Ser Thr Gln Ala Gly His Pro Ile Glu Lys Val Gly Ala Asp Leu
305 310 315 320

Arg Met Met Met Pro Phe Ile Asn Asn Asn
325 330

<210> 25
<211> 1020
<212> DNA
<213> 前庭链球菌 (Streptococcus vestibularis)

<400> 25
atggcagttc aaatggaata tgaaaaagac gtaaaagtag cagcacttga cggtaaaaaa 60

atcgccgtta tcggttacgg ttcacaaggt catgcacatg cacaaaactt gcgtgattca 120

ggtcacgatg tgatcatcgg tgttcgtcca ggtaaatcat ttgataaagc taaagaagat 180

ggttttgata cttacacagt agcagaagca actaaattgg ctgatatcat tatggttttg 240

gcaccagatg aaattcaaaa agatatctat aaagatgaaa ttgagccaaa cttgagtgca 300

ggcaaagctc ttggttttgc acacggattt aacatccact tcgaattcat caaggttcct 360

aaagatgttg atgtctttat ggttgctcct aaaggaccag gtcacttggt acgccgtaca 420

tatacagaag gctttggtgt tccagcactt tacgcagtat accaagatgc tacaggcaat 480

gctaaagaca tcgctatgga ctgggctaaa ggtattggtt ctgcacgtgt tggattactt 540

gtaacaacat ttaaagaaga aactgaagaa gatttgtttg gtgagcaagc ggttcttatg 600

ggtgggttga cacatcttat tgaagctggt tttgaagtct tgactgaagc aggctatgca 660

ccacaattgg cttactttga agtgcttcac gaaatgaaat tgatcgttga cttgatctac 720

gaaggtggat tcaagaaaat gcgtcaatca tgttcaaata ctgcagaatt tggtgacttt 780

gtaactggtc cacgtgtcat cggtccagaa gtcaaagaaa acatgaaagc tgcccttgcg 840

gatatccaat caggtaaatt tgcacgtgaa ttcgttgaag accatgatgc tggcttccca 900

cgtttgaaag cttaccgtaa agaagctgaa gagcttgaaa ttgaaaagat tggtgctgaa 960

ttgcgtaaag caatgccatt cgttggtcaa aatgacgatg acgcattcaa aatctataac 1020

<210> 26
<211> 340
<212> PRT
<213> 前庭链球菌 (Streptococcus vestibularis)

[0026]

<400> 26

Met Ala Val Gln Met Glu Tyr Glu Lys Asp Val Lys Val Ala Ala Leu
1 5 10 15

Asp Gly Lys Lys Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

His Ala Gln Asn Leu Arg Asp Ser Gly His Asp Val Ile Ile Gly Val
35 40 45

Arg Pro Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Asp Thr
50 55 60

Tyr Thr Val Ala Glu Ala Thr Lys Leu Ala Asp Ile Ile Met Val Leu
65 70 75 80

Ala Pro Asp Glu Ile Gln Lys Asp Ile Tyr Lys Asp Glu Ile Glu Pro
85 90 95

Asn Leu Ser Ala Gly Lys Ala Leu Gly Phe Ala His Gly Phe Asn Ile
100 105 110

His Phe Glu Phe Ile Lys Val Pro Lys Asp Val Asp Val Phe Met Val
115 120 125

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

Phe Gly Val Pro Ala Leu Tyr Ala Val Tyr Gln Asp Ala Thr Gly Asn
145 150 155 160

Ala Lys Asp Ile Ala Met Asp Trp Ala Lys Gly Ile Gly Ser Ala Arg
165 170 175

Val Gly Leu Leu Val Thr Thr Phe Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Met Gly Gly Leu Thr His Leu Ile Glu
195 200 205

Ala Gly Phe Glu Val Leu Thr Glu Ala Gly Tyr Ala Pro Gln Leu Ala
210 215 220

Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Ile Tyr
225 230 235 240

Glu Gly Gly Phe Lys Lys Met Arg Gln Ser Cys Ser Asn Thr Ala Glu
245 250 255

[0027]

Phe Gly Asp Phe Val Thr Gly Pro Arg Val Ile Gly Pro Glu Val Lys
260 265 270

Glu Asn Met Lys Ala Ala Leu Ala Asp Ile Gln Ser Gly Lys Phe Ala
275 280 285

Arg Glu Phe Val Glu Asp His Asp Ala Gly Phe Pro Arg Leu Lys Ala
290 295 300

Tyr Arg Lys Glu Ala Glu Glu Leu Glu Ile Glu Lys Ile Gly Ala Glu
305 310 315 320

Leu Arg Lys Ala Met Pro Phe Val Gly Gln Asn Asp Asp Asp Ala Phe
325 330 335

Lys Ile Tyr Asn
340

<210> 27
<211> 1020
<212> DNA
<213> 变异链球菌（Streptococcus mutans）

<400> 27
atggcagtag aaatgttata tgaagcagat gtaaaagtag cagcacttga tggtaagaag 60

attgctgtta ttggttatgg atcacaagga catgctcatg cccaaaactt gagagattca 120

ggacacgatg ttatcatcgg tgtgcgtcat gggaaatcgt ttgacaaggc taaagaagat 180

ggttttgata cctatgaagt gggtgaagct actaaattgg ctgatattat catggtttta 240

gcaccggatg aaattcaaaa agatatctac aaagatgaaa ttgctcctaa tttaagtgct 300

ggtaaggctc ttggttttgc tcatggtttt aatatccatt tcggttatat taaggcacca 360

gaagatgtag atgttttcat ggttgcccct aaaggaccgg gccatcttgt acgtcgtact 420

tacacagaag gatttggagt accttcttta tatgctgttt atcaaaatcc aactggtaat 480

gctgaaaata ttgctttaga ttgggctaag ggtattggtt ctgcgcgtgt aggtctcctt 540

gtaaccactt tcaaagaaga aacggaagaa gacctttttg gtgaacaagc ggttcttatg 600

ggtggtttaa ctcaccttat tgaagcaggt tttgaagtgc ttactgaagc agggtatgct 660

ccacaattgg cttattttga agtgcttcat gaaatgaaat tgattgtaga ccttatctat 720

gaaggtggtt tcaagaaaat gcgtcaatca tgttcaaata ccgctgaatt tggtgacttt 780

gtgacaggcc cacgtgttat cggtccagaa gtaaaagaaa acatgaaggc tgctcttgct 840

gatattcaat caggtaaatt tgcccgtgaa ttcgtggaag atcatgatgc tggcttccca 900

cgtttgaaag ctttccgtaa agaagctgaa ggactcgaaa tcgaaaaaat tggtgcagaa 960

cttcgtaaag caatgccatt tgttaatcaa aacgatgacg atgctttcaa aatttataac 1020

<210> 28

[0028]

<211> 340
<212> PRT
<213> 变异链球菌（Streptococcus mutans）

<400> 28

Met Ala Val Glu Met Leu Tyr Glu Ala Asp Val Lys Val Ala Ala Leu
1 5 10 15

Asp Gly Lys Lys Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

His Ala Gln Asn Leu Arg Asp Ser Gly His Asp Val Ile Ile Gly Val
35 40 45

Arg His Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Asp Thr
50 55 60

Tyr Glu Val Gly Glu Ala Thr Lys Leu Ala Asp Ile Ile Met Val Leu
65 70 75 80

Ala Pro Asp Glu Ile Gln Lys Asp Ile Tyr Lys Asp Glu Ile Ala Pro
85 90 95

Asn Leu Ser Ala Gly Lys Ala Leu Gly Phe Ala His Gly Phe Asn Ile
100 105 110

His Phe Gly Tyr Ile Lys Ala Pro Glu Asp Val Asp Val Phe Met Val
115 120 125

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

Phe Gly Val Pro Ser Leu Tyr Ala Val Tyr Gln Asn Pro Thr Gly Asn
145 150 155 160

Ala Glu Asn Ile Ala Leu Asp Trp Ala Lys Gly Ile Gly Ser Ala Arg
165 170 175

Val Gly Leu Leu Val Thr Thr Phe Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Met Gly Gly Leu Thr His Leu Ile Glu
195 200 205

Ala Gly Phe Glu Val Leu Thr Glu Ala Gly Tyr Ala Pro Gln Leu Ala
210 215 220

Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Ile Tyr
225 230 235 240

[0029]

Glu Gly Gly Phe Lys Lys Met Arg Gln Ser Cys Ser Asn Thr Ala Glu
245 250 255

Phe Gly Asp Phe Val Thr Gly Pro Arg Val Ile Gly Pro Glu Val Lys
260 265 270

Glu Asn Met Lys Ala Ala Leu Ala Asp Ile Gln Ser Gly Lys Phe Ala
275 280 285

Arg Glu Phe Val Glu Asp His Asp Ala Gly Phe Pro Arg Leu Lys Ala
290 295 300

Phe Arg Lys Glu Ala Glu Gly Leu Glu Ile Glu Lys Ile Gly Ala Glu
305 310 315 320

Leu Arg Lys Ala Met Pro Phe Val Asn Gln Asn Asp Asp Asp Ala Phe
325 330 335

Lys Ile Tyr Asn
340

<210> 29
<211> 1020
<212> DNA
<213> 格登链球菌 (Streptococcus gordonii)

<400> 29
atggcagttc aaatggaata cgaaaaagat gtaaaagtag cagcgcttga cggtaagaaa 60

attgccgtaa tcggctatgg ttcacaaggt catgctcatg cacaaaatct gcgtgataca 120

ggccatgatg tgattatcgg tgttcgccct ggtaagtcat tcgataaggc taaagaagat 180

ggctttgata cttacacagt agcagaggca gctaagttgg ctgatgtaat catgattttg 240

gcgccagatg aaattcaaca agaactctac gaagcagaaa tcgctccaaa cttggaagct 300

ggtaatgcag ttggatttgc ccatggtttc aatatccatt ttgaatttat caaagttcct 360

gctgatgtag atgtctttat gtgtgcccct aaaggtcctg gtcacttggt tcgtcgtact 420

tttgaagaag gatttggtgt tccggctctt tatgcagtct accaagatgc aacaggaaat 480

gcgaaaaaca ttgctatgga ctggtgtaaa ggtgttggtg cagctcgtgt tggtttgctt 540

gaaacaactt acaaagaaga aacagaagaa gatctctttg gtgaacaagc tgtcctttgt 600

ggtggtttga ctgcccttat cgaagcaggt tttgaagtct tgacagaagc aggttacgct 660

cctgaattgg cttactttga agttcttcat gaaatgaaat tgatcgttga cttgatctac 720

gagggtggat tcaagaaaat gcgccaatca atttcaaata ctgctgaata tggtgattac 780

gtatcaggcc cacgtgtaat tactgagcaa gtcaaagaaa acatgaaggc tgttttggca 840

gacattcaaa atggtaaatt tgcaaatgac tttgtaaatg actacaaggc tggccgtcca 900

aaactcactg cttaccgtga acaagcagcc aaccttgaaa tcgaaaaagt aggtgctgaa 960

[0030]

ttgcgtaaag caatgccatt tgttggtaaa aatgacgacg acgcatttaa aatctacaac 1020

<210> 30
<211> 340
<212> PRT
<213> 格登链球菌 (Streptococcus gordonii)

<400> 30

Met Ala Val Gln Met Glu Tyr Glu Lys Asp Val Lys Val Ala Ala Leu
1 5 10 15

Asp Gly Lys Lys Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

His Ala Gln Asn Leu Arg Asp Thr Gly His Asp Val Ile Ile Gly Val
35 40 45

Arg Pro Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Asp Thr
50 55 60

Tyr Thr Val Ala Glu Ala Ala Lys Leu Ala Asp Val Ile Met Ile Leu
65 70 75 80

Ala Pro Asp Glu Ile Gln Gln Glu Leu Tyr Glu Ala Glu Ile Ala Pro
85 90 95

Asn Leu Glu Ala Gly Asn Ala Val Gly Phe Ala His Gly Phe Asn Ile
100 105 110

His Phe Glu Phe Ile Lys Val Pro Ala Asp Val Asp Val Phe Met Cys
115 120 125

Ala Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Glu Glu Gly
130 135 140

Phe Gly Val Pro Ala Leu Tyr Ala Val Tyr Gln Asp Ala Thr Gly Asn
145 150 155 160

Ala Lys Asn Ile Ala Met Asp Trp Cys Lys Gly Val Gly Ala Ala Arg
165 170 175

Val Gly Leu Leu Glu Thr Thr Tyr Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Ile Glu
195 200 205

Ala Gly Phe Glu Val Leu Thr Glu Ala Gly Tyr Ala Pro Glu Leu Ala
210 215 220

[0031]

Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Ile Tyr
225 230 235 240

Glu Gly Gly Phe Lys Lys Met Arg Gln Ser Ile Ser Asn Thr Ala Glu
245 250 255

Tyr Gly Asp Tyr Val Ser Gly Pro Arg Val Ile Thr Glu Gln Val Lys
260 265 270

Glu Asn Met Lys Ala Val Leu Ala Asp Ile Gln Asn Gly Lys Phe Ala
275 280 285

Asn Asp Phe Val Asn Asp Tyr Lys Ala Gly Arg Pro Lys Leu Thr Ala
290 295 300

Tyr Arg Glu Gln Ala Ala Asn Leu Glu Ile Glu Lys Val Gly Ala Glu
305 310 315 320

Leu Arg Lys Ala Met Pro Phe Val Gly Lys Asn Asp Asp Asp Ala Phe
325 330 335

Lys Ile Tyr Asn
340

<210> 31
<211> 1020
<212> DNA
<213> 猪链球菌 (Streptococcus suis)

<400> 31
atgacagtaa caatgcaata tgaaaaagat gtaacagtgg cagcacttga cggtaaacgt 60

atcgccgtta tcggttatgg ttcacaaggt catgcccatg ctcaaaactt gcgtgataca 120

ggacacgatg tcatcatcgg tgtgcgtgca ggtaagtcat ttgacaaggc aaaagaagac 180

ggttttgaaa cttttgaagt agcagaggca gcaaaacaag ctgatgtcat tatgattttg 240

gctccagacg aaattcaagc agatttgtat gagaacgaag ttgctccaaa cttggaagct 300

ggaaatgcgc ttggttttgc tcatggtttc aacgttcact ttgaatttat caaggtacca 360

gcagatgtgg atgtcttcat gtgtgcacca aaaggtccag gtcacttggt ccgccgtacc 420

tttgaagaag gttttggagt accagccctc tatgcggtct accaagatgc aactggcaat 480

gcaaaacata ttgcgatgga ctgggcaaaa ggtgttggtt cagcccgtgt tggtcttttg 540

gaaacaactt tcaaagaaga gacagaagaa gatttgtttg gtgagcaagc tgttctctgc 600

ggtggtttga cagcccttat gcaggcaggt tttgaagtct tgacagaagc tggttatgca 660

ccagaattgg cttatttcga agttctccac gagatgaaac tcatcgttga ccttgtctac 720

gaaggtggct ttaagaaaat gcgccaatct atttcaaaca ctgctgaatt tggtgactat 780

gtatcaggtc cacgcgtgat tacagaccaa gtcaaagaaa acatgaaggc agtccttgca 840

[0032]

gatattcaat ctggtaaatt tgcaaatgac tttgtaaatg actataaagc aggccgtcca 900

cgtatggaag cttatcgtaa agaagcagag aatcttgaaa tcgaaaaagt aggtgcagaa 960

ctccgcaaag caatgccatt tgtcggacgc aatgatgacg acgcattcaa aatctataat 1020

<210> 32
<211> 340
<212> PRT
<213> 猪链球菌 (Streptococcus suis)

<400> 32

Met Thr Val Thr Met Gln Tyr Glu Lys Asp Val Thr Val Ala Ala Leu
1 5 10 15

Asp Gly Lys Arg Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

His Ala Gln Asn Leu Arg Asp Thr Gly His Asp Val Ile Ile Gly Val
35 40 45

Arg Ala Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Glu Thr
50 55 60

Phe Glu Val Ala Glu Ala Ala Lys Gln Ala Asp Val Ile Met Ile Leu
65 70 75 80

Ala Pro Asp Glu Ile Gln Ala Asp Leu Tyr Glu Asn Glu Val Ala Pro
85 90 95

Asn Leu Glu Ala Gly Asn Ala Leu Gly Phe Ala His Gly Phe Asn Val
100 105 110

His Phe Glu Phe Ile Lys Val Pro Ala Asp Val Asp Val Phe Met Cys
115 120 125

Ala Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Glu Glu Gly
130 135 140

Phe Gly Val Pro Ala Leu Tyr Ala Val Tyr Gln Asp Ala Thr Gly Asn
145 150 155 160

Ala Lys His Ile Ala Met Asp Trp Ala Lys Gly Val Gly Ser Ala Arg
165 170 175

Val Gly Leu Leu Glu Thr Thr Phe Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Met Gln
195 200 205

[0033]

Ala Gly Phe Glu Val Leu Thr Glu Ala Gly Tyr Ala Pro Glu Leu Ala
210 215 220

Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Val Tyr
225 230 235 240

Glu Gly Gly Phe Lys Lys Met Arg Gln Ser Ile Ser Asn Thr Ala Glu
245 250 255

Phe Gly Asp Tyr Val Ser Gly Pro Arg Val Ile Thr Asp Gln Val Lys
260 265 270

Glu Asn Met Lys Ala Val Leu Ala Asp Ile Gln Ser Gly Lys Phe Ala
275 280 285

Asn Asp Phe Val Asn Asp Tyr Lys Ala Gly Arg Pro Arg Met Glu Ala
290 295 300

Tyr Arg Lys Glu Ala Glu Asn Leu Glu Ile Glu Lys Val Gly Ala Glu
305 310 315 320

Leu Arg Lys Ala Met Pro Phe Val Gly Arg Asn Asp Asp Asp Ala Phe
325 330 335

Lys Ile Tyr Asn
340

<210> 33
<211> 1020
<212> DNA
<213> 婴儿链球菌（Streptococcus infantarius）

<400> 33
atggcagtaa caatggaata cgaaaaagac gtaaaagtag cagctcttga tggtaaaaaa 60

attgccgtta ttggttatgg atcacaaggt catgctcatg ctcaaaactt gcgtgactca 120

ggtcacgatg ttatcattgg ggttcgccat ggtaaatcat tcgataaagc aaaagaagat 180

ggatttgata cttatgaagt agcagaagca acaaaacttg ctgatgttat catggtattg 240

gctcctgatg aaatccaagc taaactttat gctgaagaaa tcgctccaaa ccttgaagct 300

ggtaacgctc ttggatttgc acatggtttt aatatccgtt ttggatacat taaagctcca 360

gaaacagtag atgtcttcat gtgtgctcct aaaggaccag gtcaccttgt tcgtcgtact 420

tacacagaag gatttggtgt accagcactt tacgctgttt accaagatgc tactggtaat 480

gctaaagaca tcgcaatgga ttggtctaaa ggtatcggtg ctgcacgtgt tggacttctt 540

gaaacaacat ttaaagaaga aactgaagaa gacctctttg gtgaacaagc agtactttgt 600

ggtggtttaa ctgctcttat cgaagctggt tttgaagttc ttactgaagc tggctatgct 660

ccagaattgg cttactttga agttcttcat gaaatgaaac ttatcgttga ccttatctac 720

[0034]

gaaggtggat tcaagaaaat gcgtcaatca atttcaaata cagctgaatt tggtgactac 780

gtatctggac cacgtgttat cactaaagat gttaaagaaa atatgaaagc tgttcttgct 840

gatatccaat caggtaaatt cgctgaagat tttgttaacg actaccaagc aggtcgtcca 900

aaacttgaag cataccgtaa agaagctgca gctcttgaaa ttgaaaaagt gggtgctgaa 960

cttcgtaaag caatgccttt tgttaaccaa aacgatgacg atgcattcaa aatttataac 1020

<210> 34
<211> 340
<212> PRT
<213> 婴儿链球菌 (Streptococcus infantarius)

<400> 34

Met Ala Val Thr Met Glu Tyr Glu Lys Asp Val Lys Val Ala Ala Leu
1 5 10 15

Asp Gly Lys Lys Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

His Ala Gln Asn Leu Arg Asp Ser Gly His Asp Val Ile Ile Gly Val
35 40 45

Arg His Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Asp Thr
50 55 60

Tyr Glu Val Ala Glu Ala Thr Lys Leu Ala Asp Val Ile Met Val Leu
65 70 75 80

Ala Pro Asp Glu Ile Gln Ala Lys Leu Tyr Ala Glu Glu Ile Ala Pro
85 90 95

Asn Leu Glu Ala Gly Asn Ala Leu Gly Phe Ala His Gly Phe Asn Ile
100 105 110

Arg Phe Gly Tyr Ile Lys Ala Pro Glu Thr Val Asp Val Phe Met Cys
115 120 125

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

Phe Gly Val Pro Ala Leu Tyr Ala Val Tyr Gln Asp Ala Thr Gly Asn
145 150 155 160

Ala Lys Asp Ile Ala Met Asp Trp Ser Lys Gly Ile Gly Ala Ala Arg
165 170 175

Val Gly Leu Leu Glu Thr Thr Phe Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

[0035]

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Ile Glu
195 200 205

Ala Gly Phe Glu Val Leu Thr Glu Ala Gly Tyr Ala Pro Glu Leu Ala
210 215 220

Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Ile Tyr
225 230 235 240

Glu Gly Gly Phe Lys Lys Met Arg Gln Ser Ile Ser Asn Thr Ala Glu
245 250 255

Phe Gly Asp Tyr Val Ser Gly Pro Arg Val Ile Thr Lys Asp Val Lys
260 265 270

Glu Asn Met Lys Ala Val Leu Ala Asp Ile Gln Ser Gly Lys Phe Ala
275 280 285

Glu Asp Phe Val Asn Asp Tyr Gln Ala Gly Arg Pro Lys Leu Glu Ala
290 295 300

Tyr Arg Lys Glu Ala Ala Ala Leu Glu Ile Glu Lys Val Gly Ala Glu
305 310 315 320

Leu Arg Lys Ala Met Pro Phe Val Asn Gln Asn Asp Asp Asp Ala Phe
325 330 335

Lys Ile Tyr Asn
340

<210> 35
<211> 1020
<212> DNA
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 35
atggcagtta caatgtatta tgaagaagat gtagaagtag ccgcactcgc gggtaagaaa 60

atcgcagtga ttggatatgg ctcacaagga cacgctcatg cacaaaactt gcgtgattct 120

ggtcatgatg tgattattgg tgtccgtcag gggaaatctt ttgataaagc aaaagaagat 180

ggttttgaaa catttgaagt aggagaagca gtagctaaag ctgacgtcat tatggttctg 240

gcacctgatg aacttcaaca atctatttat gaagaggaca taaaaccaaa tttgaaagca 300

ggttcagcac ttggttttgc ccatggtttc aatattcatt ttggctatat tgaagttcca 360

gaagatgttg atgtcttcat ggttgcgcca aaagcgccgg gacatctcgt tcggcggact 420

tttaccgaag gtttcggaac gccagctttg ttcgtttcgc atcaaaatgc cactggtcat 480

gcgcgtgaaa ttgccatgga ctgggccaaa ggaattggct gtgcccgtgt cggtatcatt 540

gaaacaactt tcaaagaaga aacagaagaa gatttgtttg gcgaacaggc cgtgctttgt 600

[0036]

ggcggtttga cagcacttgt tgaagctggt tttgaaacac tgacagaagc tggatatgct 660

ggcgaattgg cttactttga agtgctgcat gaaatgaaat tgattgttga ccttatgtac 720

gaaggtggtt tcactaaaat gcgtcagtca atctcaaaca ctgccgaatt tggtgattat 780

gtgactggac cacgcattat tactgacgaa gttaaaaaga atatgaaact cgtgttggct 840

gacattcaat caggaaaatt tgcgcaagat ttcgttgatg atttcaaagc tggacgtcca 900

aaattaactg cttatcgtga agcagctaaa aatctggaaa ttgaaaaaat cggtgcagaa 960

ctacgtaaag caatgccatt tacacaatct ggtgatgacg acgcctttaa aatttatcaa 1020

<210> 36
<211> 340
<212> PRT
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 36

Met Ala Val Thr Met Tyr Tyr Glu Glu Asp Val Glu Val Ala Ala Leu
1 5 10 15

Ala Gly Lys Lys Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

His Ala Gln Asn Leu Arg Asp Ser Gly His Asp Val Ile Ile Gly Val
35 40 45

Arg Gln Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Glu Thr
50 55 60

Phe Glu Val Gly Glu Ala Val Ala Lys Ala Asp Val Ile Met Val Leu
65 70 75 80

Ala Pro Asp Glu Leu Gln Gln Ser Ile Tyr Glu Glu Asp Ile Lys Pro
85 90 95

Asn Leu Lys Ala Gly Ser Ala Leu Gly Phe Ala His Gly Phe Asn Ile
100 105 110

His Phe Gly Tyr Ile Glu Val Pro Glu Asp Val Asp Val Phe Met Val
115 120 125

Ala Pro Lys Ala Pro Gly His Leu Val Arg Arg Thr Phe Thr Glu Gly
130 135 140

Phe Gly Thr Pro Ala Leu Phe Val Ser His Gln Asn Ala Thr Gly His
145 150 155 160

Ala Arg Glu Ile Ala Met Asp Trp Ala Lys Gly Ile Gly Cys Ala Arg
165 170 175

[0037]

Val Gly Ile Ile Glu Thr Thr Phe Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Val Glu
195 200 205

Ala Gly Phe Glu Thr Leu Thr Glu Ala Gly Tyr Ala Gly Glu Leu Ala
210 215 220

Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr
225 230 235 240

Glu Gly Gly Phe Thr Lys Met Arg Gln Ser Ile Ser Asn Thr Ala Glu
245 250 255

Phe Gly Asp Tyr Val Thr Gly Pro Arg Ile Ile Thr Asp Glu Val Lys
260 265 270

Lys Asn Met Lys Leu Val Leu Ala Asp Ile Gln Ser Gly Lys Phe Ala
275 280 285

Gln Asp Phe Val Asp Asp Phe Lys Ala Gly Arg Pro Lys Leu Thr Ala
290 295 300

Tyr Arg Glu Ala Ala Lys Asn Leu Glu Ile Glu Lys Ile Gly Ala Glu
305 310 315 320

Leu Arg Lys Ala Met Pro Phe Thr Gln Ser Gly Asp Asp Asp Ala Phe
325 330 335

Lys Ile Tyr Gln
340

<210> 37
<211> 1032
<212> DNA
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 37
atggcagtta caatgtatta tgaagatgat gtagaagtat cagcacttgc tggaaagcaa 60

attgcagtaa tcggttatgg ttcacaagga catgctcacg cacagaattt gcgtgattct 120

ggtcacaacg ttatcattgg tgtgcgccac ggaaaatctt ttgataaagc aaaagaagat 180

ggctttgaaa catttgaagt aggagaagca gtagctaaag ctgatgttat tatggttttg 240

gcaccagatg aacttcaaca atccatttat gaagaggaca tcaaaccaaa cttgaaagca 300

ggttcagcac ttggttttgc tcacggattt aatatccatt ttggctatat taaagtacca 360

gaagacgttg acgtctttat ggttgcgcct aaggctccag gtcaccttgt ccgtcggact 420

tatactgaag gttttggtac accagctttg tttgtttcac accaaaatgc aagtggtcat 480

[0038]

gcgcgtgaaa tcgcaatgga ttgggccaaa ggaattggtt gtgctcgagt gggaattatt 540

gaaacaactt ttaaagaaga aacagaagaa gatttgtttg gagaacaagc tgttctatgt 600

ggaggtttga cagcacttgt tgaagccggt tttgaaacac tgacagaagc tggatacgct 660

ggcgaattgg cttactttga agttttgcac gaaatgaaat tgattgttga cctcatgtat 720

gaaggtggtt ttactaaaat gcgtcaatcc atctcaaata ctgctgagtt tggcgattat 780

gtgactggtc cacggattat tactgacgaa gttaaaaaga atatgaagct tgttttggct 840

gatattcaat ctggaaaatt tgctcaagat ttcgttgatg acttcaaagc ggggcgtcca 900

aaattaatag cctatcgcga agctgcaaaa aatcttgaaa ttgaaaaaat tggggcagag 960

cacgtcaagc aatgccattc acacaatctg gtgatgacga tgcctttaaa atctatcagt 1020

aatttctctt at 1032

<210> 38
<211> 344
<212> PRT
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 38

Met Ala Val Thr Met Tyr Tyr Glu Asp Asp Val Glu Val Ser Ala Leu
1 5 10 15

Ala Gly Lys Gln Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

His Ala Gln Asn Leu Arg Asp Ser Gly His Asn Val Ile Ile Gly Val
35 40 45

Arg His Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Glu Thr
50 55 60

Phe Glu Val Gly Glu Ala Val Ala Lys Ala Asp Val Ile Met Val Leu
65 70 75 80

Ala Pro Asp Glu Leu Gln Gln Ser Ile Tyr Glu Glu Asp Ile Lys Pro
85 90 95

Asn Leu Lys Ala Gly Ser Ala Leu Gly Phe Ala His Gly Phe Asn Ile
100 105 110

His Phe Gly Tyr Ile Lys Val Pro Glu Asp Val Asp Val Phe Met Val
115 120 125

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

Phe Gly Thr Pro Ala Leu Phe Val Ser His Gln Asn Ala Ser Gly His
145 150 155 160

[0039]

Ala Arg Glu Ile Ala Met Asp Trp Ala Lys Gly Ile Gly Cys Ala Arg
165 170 175

Val Gly Ile Ile Glu Thr Thr Phe Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Val Glu
195 200 205

Ala Gly Phe Glu Thr Leu Thr Glu Ala Gly Tyr Ala Gly Glu Leu Ala
210 215 220

Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr
225 230 235 240

Glu Gly Gly Phe Thr Lys Met Arg Gln Ser Ile Ser Asn Thr Ala Glu
245 250 255

Phe Gly Asp Tyr Val Thr Gly Pro Arg Ile Ile Thr Asp Glu Val Lys
260 265 270

Lys Asn Met Lys Leu Val Leu Ala Asp Ile Gln Ser Gly Lys Phe Ala
275 280 285

Gln Asp Phe Val Asp Asp Phe Lys Ala Gly Arg Pro Lys Leu Ile Ala
290 295 300

Tyr Arg Glu Ala Ala Lys Asn Leu Glu Ile Glu Lys Ile Gly Ala Glu
305 310 315 320

His Val Lys Gln Cys His Ser His Asn Leu Val Met Thr Met Pro Leu
325 330 335

Lys Ser Ile Ser Asn Phe Ser Tyr
340

<210> 39
<211> 1041
<212> DNA
<213> 肠膜明串珠菌（Leuconcstoc mesenteroides）

<400> 39
atgactacaa aaatgtttta tgataaagat attgatacaa caccattgga aaacaaaaag 60

attgcagtta ttggctatgg tgcacaaggg catgctcaag cgaataatct acgcgactca 120

ggatttgatg tcatcatggg attgcgccca ggaaaatctt ttgatagtgc taaaaaagat 180

gggttcgaag tttactcagc tgctgaagca acggcacaag cggatgttgt tatgatggaa 240

acccctgacg aattacaagc ggcagtttgg gagaaagaag ttgagcctaa ccttaaggca 300

[0040]

ggttcttacc ttggattttc tcatgggttc aacattgttt atggtttgat taagcccaat 360

gctgatatta atgtcatgat cattgcgcca aagggtccag gaaacattga acgtcgccaa 420

ttcgttgaag ggggtgggat tccttctttg tatggtgttc atcaagatcc tacgggtgat 480

acagctgaag tggccaaggc ctatgcgaaa ggtattggtt caggtcgcgc aggtatcttg 540

gaaacgactt ttgaagaaga aacaacagaa gacttgtttg gtgaacaagc tgtactttgt 600

ggtggcttaa cacaattgat tgaggcagga tttaacacat tggtggaagc aggttactca 660

cctgaattgg cttatttcga aacatctcat gaaatgaaga tgattgttga tttgatcttt 720

gaaggtggtt tcgagaagat gagacacgat tgctcaaata cttgtgaata tggtgaaatg 780

ttgaacggac cacgtatcat cactgaagaa tcaaagcaag gaatgcgtga tgttttgaag 840

gatatccaag atggtactta tgctaagaag tggttggcag aatacaattc tggtttgaag 900

gatttggaaa agatgcggac agagtataag agcggtttgt acgagcaaac aggtaagaag 960

gttcgtgcta tgatgccatg gatttcagat gcagataagt actcgacagc agcagatact 1020

gagcaattct cagcagcaaa g 1041

<210> 40
<211> 347
<212> PRT
<213> 人葡萄球菌（Staphylococcus hominis）

<400> 40

Met Thr Thr Lys Met Phe Tyr Asp Lys Asp Ile Asp Thr Thr Pro Leu
1 5 10 15

Glu Asn Lys Lys Ile Ala Val Ile Gly Tyr Gly Ala Gln Gly His Ala
20 25 30

Gln Ala Asn Asn Leu Arg Asp Ser Gly Phe Asp Val Ile Met Gly Leu
35 40 45

Arg Pro Gly Lys Ser Phe Asp Ser Ala Lys Lys Asp Gly Phe Glu Val
50 55 60

Tyr Ser Ala Ala Glu Ala Thr Ala Gln Ala Asp Val Val Met Met Glu
65 70 75 80

Thr Pro Asp Glu Leu Gln Ala Ala Val Trp Glu Lys Glu Val Glu Pro
85 90 95

Asn Leu Lys Ala Gly Ser Tyr Leu Gly Phe Ser His Gly Phe Asn Ile
100 105 110

Val Tyr Gly Leu Ile Lys Pro Asn Ala Asp Ile Asn Val Met Ile Ile
115 120 125

[0041]

Ala Pro Lys Gly Pro Gly Asn Ile Glu Arg Arg Gln Phe Val Glu Gly
130 135 140

Gly Gly Ile Pro Ser Leu Tyr Gly Val His Gln Asp Pro Thr Gly Asp
145 150 155 160

Thr Ala Glu Val Ala Lys Ala Tyr Ala Lys Gly Ile Gly Ser Gly Arg
165 170 175

Ala Gly Ile Leu Glu Thr Thr Phe Glu Glu Glu Thr Thr Glu Asp Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Gln Leu Ile Glu
195 200 205

Ala Gly Phe Asn Thr Leu Val Glu Ala Gly Tyr Ser Pro Glu Leu Ala
210 215 220

Tyr Phe Glu Thr Ser His Glu Met Lys Met Ile Val Asp Leu Ile Phe
225 230 235 240

Glu Gly Gly Phe Glu Lys Met Arg His Asp Cys Ser Asn Thr Cys Glu
245 250 255

Tyr Gly Glu Met Leu Asn Gly Pro Arg Ile Ile Thr Glu Glu Ser Lys
260 265 270

Gln Gly Met Arg Asp Val Leu Lys Asp Ile Gln Asp Gly Thr Tyr Ala
275 280 285

Lys Lys Trp Leu Ala Glu Tyr Asn Ser Gly Leu Lys Asp Leu Glu Lys
290 295 300

Met Arg Thr Glu Tyr Lys Ser Gly Leu Tyr Glu Gln Thr Gly Lys Lys
305 310 315 320

Val Arg Ala Met Met Pro Trp Ile Ser Asp Ala Asp Lys Tyr Ser Thr
325 330 335

Ala Ala Asp Thr Glu Gln Phe Ser Ala Ala Lys
340 345

<210> 41
<211> 1044
<212> DNA
<213> 布氏乳杆菌 (Lactobacillus buchneri)

<400> 41
atgagtgtag aaatgttgta tgacaaggat gttaccacca ttatcttca aggaaagaaa 60

attgctttta tcggttatgg atcacaaggt catgcccaag ctaacaactt aagagattca 120

[0042]

ggttatgacg ttatcgttgg tgttcgccca ggacaatcat ttgaaaatgc caaagttgat 180

ggatttgatg tatacacacc agccgaagca gctcgtcgtg cagactggat tcaaatgtta 240

acgcctgatg aagttatggg cgacgtttat aaaaatgaaa ttgcacctaa cctagaggaa 300

ggcaatgtat taggcttttc acatggcttc aacattcatt ataaagaaat cgtaccacca 360

gcaaacgttg atgttgttat gatggcacct aaaggcccag gtaatctttg tcgtcgtaca 420

tacgttgaag gctccggtgt tccagcatta tatggctatt tccaagacta ttcaggtcat 480

gccgaagatt tatccaagga attcgccaaa ggaaatggtg cagcacgtgc cggactattg 540

aagacaacct tcaaagaaga aactgaagaa gatttgtttg gtgagcaaaa cgtccttatg 600

ggtggtgtta ccgcgcttat cgaaaccggt tatgaagtct taaccgaagc aggatactca 660

ccacagttgg catacttcga agtggatcac gaaatgaaat tgatctgtga ccttatctac 720

gaaggtgggt tcaacaagat gtatgctgac tgctcgaaca cttctgaata tggttcatat 780

gtagttggtc caaaggtcgt tggtaaggaa tccaagcaag ctatgaaaga cgctttgaag 840

cggattcaag acggttcatt tgctaaagaa ttcatggatg actaccgtaa cggcttcaag 900

aagctttaca agatgcgtga acgttcagct aactcacttc tttcacgtgt tggtgctgat 960

cttcgtgatc acatgtcatt tgttggtgaa gccgacaagt acagtacacc tactgaagaa 1020

aaagcagaag cagaagctga aaag 1044

<210> 42
<211> 348
<212> PRT
<213> 布氏乳杆菌 (Lactobacillus buchneri)

<400> 42

Met Ser Val Glu Met Leu Tyr Asp Lys Asp Val Thr Thr Asn Tyr Leu
1 5 10 15

Gln Gly Lys Lys Ile Ala Phe Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

Gln Ala Asn Asn Leu Arg Asp Ser Gly Tyr Asp Val Ile Val Gly Val
35 40 45

Arg Pro Gly Gln Ser Phe Glu Asn Ala Lys Val Asp Gly Phe Asp Val
50 55 60

Tyr Thr Pro Ala Glu Ala Ala Arg Arg Ala Asp Trp Ile Gln Met Leu
65 70 75 80

Thr Pro Asp Glu Val Met Gly Asp Val Tyr Lys Asn Glu Ile Ala Pro
85 90 95

Asn Leu Glu Glu Gly Asn Val Leu Gly Phe Ser His Gly Phe Asn Ile
100 105 110

[0043]

His Tyr Lys Glu Ile Val Pro Pro Ala Asn Val Asp Val Val Met Met
115 120 125

Ala Pro Lys Gly Pro Gly Asn Leu Cys Arg Arg Thr Tyr Val Glu Gly
130 135 140

Ser Gly Val Pro Ala Leu Tyr Gly Tyr Phe Gln Asp Tyr Ser Gly His
145 150 155 160

Ala Glu Asp Leu Ser Lys Glu Phe Ala Lys Gly Asn Gly Ala Ala Arg
165 170 175

Ala Gly Leu Leu Lys Thr Thr Phe Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

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

Thr Gly Tyr Glu Val Leu Thr Glu Ala Gly Tyr Ser Pro Gln Leu Ala
210 215 220

Tyr Phe Glu Val Asp His Glu Met Lys Leu Ile Cys Asp Leu Ile Tyr
225 230 235 240

Glu Gly Gly Phe Asn Lys Met Tyr Ala Asp Cys Ser Asn Thr Ser Glu
245 250 255

Tyr Gly Ser Tyr Val Val Gly Pro Lys Val Val Gly Lys Glu Ser Lys
260 265 270

Gln Ala Met Lys Asp Ala Leu Lys Arg Ile Gln Asp Gly Ser Phe Ala
275 280 285

Lys Glu Phe Met Asp Asp Tyr Arg Asn Gly Phe Lys Lys Leu Tyr Lys
290 295 300

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

Leu Arg Asp His Met Ser Phe Val Gly Glu Ala Asp Lys Tyr Ser Thr
325 330 335

Pro Thr Glu Glu Lys Ala Glu Ala Glu Ala Glu Lys
340 345

<210> 43
<211> 1002
<212> DNA
<213> 溶血性葡萄球菌 (Staphylococcus haemolyticus)

[0044]

<400> 43
atgactaaag tttattacga tcaatcagtt gagaaagatg cattacaagg taaaaaaatt 60

gcaattatcg gttacggttc acaaggacac gcgcatgcac aaaaccttaa agacaacggt 120

tatgacgtta ttgttggtat tcgccctggt cattcttttg ataaagctaa agaagatggc 180

ttcgatgttt atccagttgc agaagctgtt aaacaagcag atgtcatcat ggtgttattg 240

ccagatgaaa tccaaggaaa tgtctataaa aatgaaattg aacctaactt agaagctgga 300

aatgccctag catttgcgca tggttttaat atccactttg aagttatcaa accacctaaa 360

gatgtggatg tcttcttagt agctcctaaa ggacctggtc atttagtaag acgtaccttt 420

gttgaaggaa ctgccgtacc agcactattt ggtgtacaac aagatgctac tggtcaagca 480

cgcgatattt cattaagtta tgcgaaaggt atcggagcta cgcgtgcagg tgtaattgaa 540

acaacattta aagaagaaac tgaaacagat ttattcggtg agcaagcagt actttgtggt 600

ggtattcata aattaatcca aagtggcttt gaaactttag ttgaagcggg ctatcaaaaa 660

gaattagcat acttcgaagt attacatgaa atgaagttaa ttgttgattt aatgtatgaa 720

ggcggtatgg agaacgttcg ttactcaatt tctaacacag cagagtatgg tgactatgtt 780

tcaggaccta gagtcatcac tcctgatgtt aaagacaata tgaaagcagt attaaaagat 840

attcaaaatg gtaattttgc aaatagcttt gttaaagaca atgaaaatgg ctttaaagaa 900

ttctataaat tacgtgaaca acaacacggt catgaaatcg aagcagttgg tcgcgaatta 960

agaaaaatga tgccattcat taaatctaaa agtattcaaa aa 1002

<210> 44
<211> 334
<212> PRT
<213> 溶血性葡萄球菌 (Staphylococcus haemolyticus)

<400> 44

Met Thr Lys Val Tyr Tyr Asp Gln Ser Val Glu Lys Asp Ala Leu Gln
1 5 10 15

Gly Lys Lys Ile Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala His
20 25 30

Ala Gln Asn Leu Lys Asp Asn Gly Tyr Asp Val Ile Val Gly Ile Arg
35 40 45

Pro Gly His Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Asp Val Tyr
50 55 60

Pro Val Ala Glu Ala Val Lys Gln Ala Asp Val Ile Met Val Leu Leu
65 70 75 80

Pro Asp Glu Ile Gln Gly Asn Val Tyr Lys Asn Glu Ile Glu Pro Asn
85 90 95

[0045]

Leu Glu Ala Gly Asn Ala Leu Ala Phe Ala His Gly Phe Asn Ile His
100 105 110

Phe Glu Val Ile Lys Pro Pro Lys Asp Val Asp Val Phe Leu Val Ala
115 120 125

Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Val Glu Gly Thr
130 135 140

Ala Val Pro Ala Leu Phe Gly Val Gln Gln Asp Ala Thr Gly Gln Ala
145 150 155 160

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

Gly Val Ile Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Ile His Lys Leu Ile Gln Ser
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Gln Lys Glu Leu Ala Tyr
210 215 220

Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr Glu
225 230 235 240

Gly Gly Met Glu Asn Val Arg Tyr Ser Ile Ser Asn Thr Ala Glu Tyr
245 250 255

Gly Asp Tyr Val Ser Gly Pro Arg Val Ile Thr Pro Asp Val Lys Asp
260 265 270

Asn Met Lys Ala Val Leu Lys Asp Ile Gln Asn Gly Asn Phe Ala Asn
275 280 285

Ser Phe Val Lys Asp Asn Glu Asn Gly Phe Lys Glu Phe Tyr Lys Leu
290 295 300

Arg Glu Gln Gln His Gly His Glu Ile Glu Ala Val Gly Arg Glu Leu
305 310 315 320

Arg Lys Met Met Pro Phe Ile Lys Ser Lys Ser Ile Gln Lys
325 330

<210> 45
<211> 1002
<212> DNA
<213> 表皮葡萄球菌 (Staphylococcus epidermidis)

[0046]

<400> 45
atgacaaaag tatattacga cgaaacagta actcaggatg cattacaagg taaaaaaatt 60

gctgtcattg gttatggctc acaaggacat gcacatgcac aaaatttaaa ggacaatggt 120

tatgatgtag tcattggcct gcgtccagga cgatcattta ataaagctaa agaagatgga 180

tttgatgttt atacggtaag tgaagcaaca caacaagcag atgtagtgat ggtactattg 240

cctgatgaaa ttcaaggtga agtatataac aaggaaatta aaccatattt agaaaaagga 300

aatgctttag cattcgcaca cggttttaat atccatttca gtgttatcga accacctagt 360

gatgtcgatg tctttttagt agcacctaaa ggacctggtc atttagttag acgtacattt 420

gttgaaggaa gtgccgtacc agcattattt ggtgttcaac aagatgctac aggccaagct 480

agaaacattg ctttaagcta cgcaaaaggc attggtgcta ctcgtgccgg ggtcattgaa 540

acgacattta aagaagaaac tgaaacagat ttattcggtg aacaagctgt actttgtgga 600

ggagtttcca aattaattca gagtggattc gaaacacttg tggaagcagg ttaccaacct 660

gaattagctt attttgaagt cttacacgaa atgaaattaa ttgttgattt aatgtatgaa 720

ggcggaatgg aaaatgtccg ttattctatc tctaacactg ctgaatttgg cgactatgtt 780

tctggaccaa gagtaattac acctaatgtt aaagaaaata tgaaaaaagt acttgaagat 840

attcaaaatg gtaactttag ccgtagattt gttgaagata acaaaaatgg ctttaaagaa 900

ttctatcaat tacgtgaaga tcaacatggt catcaaattg aacaagttgg acgtgaatta 960

agagaaatga tgccattcat taaatctaaa agtattgaaa aa 1002

<210> 46
<211> 334
<212> PRT
<213> 表皮葡萄球菌 (Staphylococcus epidermidis)

<400> 46

Met Thr Lys Val Tyr Tyr Asp Glu Thr Val Thr Gln Asp Ala Leu Gln
1 5 10 15

Gly Lys Lys Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala His
20 25 30

Ala Gln Asn Leu Lys Asp Asn Gly Tyr Asp Val Val Ile Gly Leu Arg
35 40 45

Pro Gly Arg Ser Phe Asn Lys Ala Lys Glu Asp Gly Phe Asp Val Tyr
50 55 60

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

Pro Asp Glu Ile Gln Gly Glu Val Tyr Asn Lys Glu Ile Lys Pro Tyr
85 90 95

[0047]

Leu Glu Lys Gly Asn Ala Leu Ala Phe Ala His Gly Phe Asn Ile His
100 105 110

Phe Ser Val Ile Glu Pro Pro Ser Asp Val Asp Val Phe Leu Val Ala
115 120 125

Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Val Glu Gly Ser
130 135 140

Ala Val Pro Ala Leu Phe Gly Val Gln Gln Asp Ala Thr Gly Gln Ala
145 150 155 160

Arg Asn Ile Ala Leu Ser Tyr Ala Lys Gly Ile Gly Ala Thr Arg Ala
165 170 175

Gly Val Ile Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Val Ser Lys Leu Ile Gln Ser
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Gln Pro Glu Leu Ala Tyr
210 215 220

Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr Glu
225 230 235 240

Gly Gly Met Glu Asn Val Arg Tyr Ser Ile Ser Asn Thr Ala Glu Phe
245 250 255

Gly Asp Tyr Val Ser Gly Pro Arg Val Ile Thr Pro Asn Val Lys Glu
260 265 270

Asn Met Lys Lys Val Leu Glu Asp Ile Gln Asn Gly Asn Phe Ser Arg
275 280 285

Arg Phe Val Glu Asp Asn Lys Asn Gly Phe Lys Glu Phe Tyr Gln Leu
290 295 300

Arg Glu Asp Gln His Gly His Gln Ile Glu Gln Val Gly Arg Glu Leu
305 310 315 320

Arg Glu Met Met Pro Phe Ile Lys Ser Lys Ser Ile Glu Lys
325 330

<210> 47
<211> 1021
<212> DNA
<213> 肺炎链球菌 (Streptococcus pneumoniae)

[0048]

<400> 47
atgacagttc aaatggaata tgaaaaagat gttaaagtag cagcacttga cggtaaaaaa 60

atcgccgtta tcggttatgg ttcacaaggg catgcgcatg ctcaaaactt gcgtgattca 120

ggtcgtgacg ttattatcgg tgtacgtcca ggtaaatctt ttgataaagc aaaagaagat 180

ggatttgata cttacacagt aacagaagct actaagttgg ctgatgttat catgatcttg 240

gcgccagacg aaattcaaca agaattgtac gaagcagaaa tcgctccaaa cttggaagct 300

ggaaacgcag ttggatttgc ccatggtttc aacatccact ttgaatttat caaagttcct 360

gcggatgtag atgtcttcat gtgtgctcct aaaggaccag gacacttggt acgtcgtact 420

tacgaagaag gatttggtgt tccagctctt tatgcagtat accaagatgc aacaggaaat 480

gctaaaaaca ttgctatgga ctggtgtaaa ggtgttggag cggctcgtgt aggtcttctt 540

gaaacaactt acaaagaaga aactgaagaa gatttgtttg gtgaacaagc tgtactttgt 600

ggtggtttga ctgcccttat cgaagcaggt ttcgaagtct tgacagaagc aggttacgct 660

ccagaattgg cttactttga agttcttcac gaaatgaaat tgatcgttga cttgatctac 720

gaaggtggat tcaagaaaat gcgtcaatct atttcaaaca ctgctgaata cggtgactat 780

gtatcaggtc cacgtgtaat cactgaacaa gttaaagaaa atatgaaggc tgtcttggca 840

gacatccaaa atggtaaatt tgcaaatgac tttgtaaatg actataaagc tggacgtcca 900

aaattgactg cttaccgtga acaagcagct aaccttgaaa ttgaaaaagt tggtgcagaa 960

ttgcgtaaag caatgccatt cgttggtaaa aacgatgatg atgcattcaa aatctataac 1020

t 1021

<210> 48
<211> 340
<212> PRT
<213> 肺炎链球菌（Streptococcus pneumoniae）

<400> 48

Met Thr Val Gln Met Glu Tyr Glu Lys Asp Val Lys Val Ala Ala Leu
1 5 10 15

Asp Gly Lys Lys Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

His Ala Gln Asn Leu Arg Asp Ser Gly Arg Asp Val Ile Ile Gly Val
35 40 45

Arg Pro Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Asp Thr
50 55 60

Tyr Thr Val Thr Glu Ala Thr Lys Leu Ala Asp Val Ile Met Ile Leu
65 70 75 80

[0049]

Ala Pro Asp Glu Ile Gln Gln Glu Leu Tyr Glu Ala Glu Ile Ala Pro
85 90 95

Asn Leu Glu Ala Gly Asn Ala Val Gly Phe Ala His Gly Phe Asn Ile
100 105 110

His Phe Glu Phe Ile Lys Val Pro Ala Asp Val Asp Val Phe Met Cys
115 120 125

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

Phe Gly Val Pro Ala Leu Tyr Ala Val Tyr Gln Asp Ala Thr Gly Asn
145 150 155 160

Ala Lys Asn Ile Ala Met Asp Trp Cys Lys Gly Val Gly Ala Ala Arg
165 170 175

Val Gly Leu Leu Glu Thr Thr Tyr Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Ile Glu
195 200 205

Ala Gly Phe Glu Val Leu Thr Glu Ala Gly Tyr Ala Pro Glu Leu Ala
210 215 220

Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Ile Tyr
225 230 235 240

Glu Gly Gly Phe Lys Lys Met Arg Gln Ser Ile Ser Asn Thr Ala Glu
245 250 255

Tyr Gly Asp Tyr Val Ser Gly Pro Arg Val Ile Thr Glu Gln Val Lys
260 265 270

Glu Asn Met Lys Ala Val Leu Ala Asp Ile Gln Asn Gly Lys Phe Ala
275 280 285

Asn Asp Phe Val Asn Asp Tyr Lys Ala Gly Arg Pro Lys Leu Thr Ala
290 295 300

Tyr Arg Glu Gln Ala Ala Asn Leu Glu Ile Glu Lys Val Gly Ala Glu
305 310 315 320

Leu Arg Lys Ala Met Pro Phe Val Gly Lys Asn Asp Asp Asp Ala Phe
325 330 335

Lys Ile Tyr Asn
340

[0050]

<210> 49
<211> 1008
<212> DNA
<213> 肺炎链球菌 (Streptococcus pneumoniae)

<400> 49
atggaatatg aaaaagatgt taaagtagca gcacttgacg gtaaaaaaat cgccgttatc 60

ggttatggtt cacaagggca tgcgcatgct caaaacttgc gtgattcagg tcgtgacgtt 120

attatcggtg tacgtccagg taaatctttt gataaagcaa aagaagatgg atttgatact 180

tacacagtag cagaagctac taagttggct gatgttatca tgatcttggc gccagacgaa 240

attcaacaag aattgtacga agcagaaatc gctccaaact tggaagctgg aaacgcagtt 300

ggatttgccc atggtttcaa catccacttt gaatttatca aagttcctgc ggatgtagat 360

gtcttcatgt gtgctcctaa aggaccagga cacttggtac gtcgtactta cgaagaagga 420

tttggtgttc cagctcttta tgcagtatac caagatgcaa caggaaatgc taaaaacatt 480

gctatggact ggtgtaaagg tgttggagcg gctcgtgtag gtcttcttga aacaacttac 540

aaagaagaaa ctgaagaaga tttgtttggt gaacaagctg tactttgtgg tggtttgact 600

gcccttatcg aagcaggttt cgaagtcttg acagaagcag gttacgctcc agaattggct 660

tactttgaag ttcttcacga aatgaaattg atcgttgact tgatctacga aggtggattc 720

aagaaaatgc gtcaatctat ttcaaacact gctgaatacg gtgactatgt atcaggtcca 780

cgtgtaatca ctgaacaagt taaagaaaat atgaaggctg tcttggcaga catccaaaat 840

ggtaaatttg caaatgactt tgtaaatgac tataaagctg gacgtccaaa attgactgct 900

taccgtgaac aagcagctaa ccttgaaatt gaaaaagttg gtgcagaatt gcgtaaagca 960

atgccattcg ttggtaaaaa cgacgatgat gcattcaaaa tctataac 1008

<210> 50
<211> 336
<212> PRT
<213> 肺炎链球菌 (Streptococcus pneumoniae)

<400> 50

Met Glu Tyr Glu Lys Asp Val Lys Val Ala Ala Leu Asp Gly Lys Lys
1 5 10 15

Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala His Ala Gln Asn
20 25 30

Leu Arg Asp Ser Gly Arg Asp Val Ile Ile Gly Val Arg Pro Gly Lys
35 40 45

Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Asp Thr Tyr Thr Val Ala
50 55 60

[0051]

Glu Ala Thr Lys Leu Ala Asp Val Ile Met Ile Leu Ala Pro Asp Glu
65 70 75 80

Ile Gln Gln Glu Leu Tyr Glu Ala Glu Ile Ala Pro Asn Leu Glu Ala
85 90 95

Gly Asn Ala Val Gly Phe Ala His Gly Phe Asn Ile His Phe Glu Phe
100 105 110

Ile Lys Val Pro Ala Asp Val Asp Val Phe Met Cys Ala Pro Lys Gly
115 120 125

Pro Gly His Leu Val Arg Arg Thr Tyr Glu Glu Gly Phe Gly Val Pro
130 135 140

Ala Leu Tyr Ala Val Tyr Gln Asp Ala Thr Gly Asn Ala Lys Asn Ile
145 150 155 160

Ala Met Asp Trp Cys Lys Gly Val Gly Ala Ala Arg Val Gly Leu Leu
165 170 175

Glu Thr Thr Tyr Lys Glu Glu Thr Glu Glu Asp Leu Phe Gly Glu Gln
180 185 190

Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Ile Glu Ala Gly Phe Glu
195 200 205

Val Leu Thr Glu Ala Gly Tyr Ala Pro Glu Leu Ala Tyr Phe Glu Val
210 215 220

Leu His Glu Met Lys Leu Ile Val Asp Leu Ile Tyr Glu Gly Gly Phe
225 230 235 240

Lys Lys Met Arg Gln Ser Ile Ser Asn Thr Ala Glu Tyr Gly Asp Tyr
245 250 255

Val Ser Gly Pro Arg Val Ile Thr Glu Gln Val Lys Glu Asn Met Lys
260 265 270

Ala Val Leu Ala Asp Ile Gln Asn Gly Lys Phe Ala Asn Asp Phe Val
275 280 285

Asn Asp Tyr Lys Ala Gly Arg Pro Lys Leu Thr Ala Tyr Arg Glu Gln
290 295 300

Ala Ala Asn Leu Glu Ile Glu Lys Val Gly Ala Glu Leu Arg Lys Ala
305 310 315 320

Met Pro Phe Val Gly Lys Asn Asp Asp Asp Ala Phe Lys Ile Tyr Asn
325 330 335

[0052]

<210> 51
<211> 1020
<212> DNA
<213> 血链球菌 (Streptococcus sanguinis)

<400> 51
atggcagtaa caatggaata cgaaaaagat gttaaagtag cagcgcttga cggtaaaaaa 60

atcgccgtta tcggttatgg ttcacaagga catgcgcatg cacaaaactt gcgtgataca 120

ggccacgatg tgattatcgg tgttcgtcct ggtaagtcat ttgacaaggc taaagaagat 180

ggctttgata cttatacagt agcagaagca gctaaattag ctgatgtcat catgattttg 240

gctccagatg aaatccaaca agatctctat gaagcagaaa tcgctccaaa cttggaagct 300

ggaaacgcag ttgggtttgc tcatggtttc aacatccatt ttgagtttat caaagttcct 360

gccgatgtag atgtctttat gtgtgctcct aaaggccctg gtcacttggt tcgccgtact 420

ttcgaagaag gttttggtgt accagctctg tatgcagtct accaagacgc tactggaaat 480

gccaaagaca tcgcaatgga ctggtgtaaa ggtgttggtt cagctcgtgt tggacttctt 540

gaaacaactt ataaagaaga aacagaagaa gatctctttg gtgaacaagc tgtgctttgt 600

ggtggcttga ctgccctgat tgaagcagga tttgaagttc tgactgaagc aggttatgcg 660

ccagaattgg cttactttga agtgctgcac gaaatgaaac tgattgtaga cttgatctat 720

gaaggtggct tcaagaagat gcgccaatct atctcaaata cagctgaata tggtgactat 780

gtatctggtc cgcgcgtcat tactgagcaa gtcaaagaaa acatgaaggc agtcttggct 840

gatatccaaa acggtaaatt tgcgaacgac ttcgttgatg attacaaggc tggtcgtcca 900

aaactcactg cttaccgcga gcaagctgct aatctggaga ttgaaaaggt cggtgcagaa 960

ttgcgtaaag caatgccatt cgttggtaaa aacgacgacg atgctttcaa gatttataat 1020

<210> 52
<211> 340
<212> PRT
<213> 血链球菌 (Streptococcus sanguinis)

<400> 52

Met Ala Val Thr Met Glu Tyr Glu Lys Asp Val Lys Val Ala Ala Leu
1 5 10 15

Asp Gly Lys Lys Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

His Ala Gln Asn Leu Arg Asp Thr Gly His Asp Val Ile Ile Gly Val
35 40 45

Arg Pro Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Asp Thr
50 55 60

[0053]

Tyr Thr Val Ala Glu Ala Ala Lys Leu Ala Asp Val Ile Met Ile Leu
65 70 75 80

Ala Pro Asp Glu Ile Gln Gln Asp Leu Tyr Glu Ala Glu Ile Ala Pro
85 90 95

Asn Leu Glu Ala Gly Asn Ala Val Gly Phe Ala His Gly Phe Asn Ile
100 105 110

His Phe Glu Phe Ile Lys Val Pro Ala Asp Val Asp Val Phe Met Cys
115 120 125

Ala Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Glu Glu Gly
130 135 140

Phe Gly Val Pro Ala Leu Tyr Ala Val Tyr Gln Asp Ala Thr Gly Asn
145 150 155 160

Ala Lys Asp Ile Ala Met Asp Trp Cys Lys Gly Val Gly Ser Ala Arg
165 170 175

Val Gly Leu Leu Glu Thr Thr Tyr Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Ile Glu
195 200 205

Ala Gly Phe Glu Val Leu Thr Glu Ala Gly Tyr Ala Pro Glu Leu Ala
210 215 220

Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Ile Tyr
225 230 235 240

Glu Gly Gly Phe Lys Lys Met Arg Gln Ser Ile Ser Asn Thr Ala Glu
245 250 255

Tyr Gly Asp Tyr Val Ser Gly Pro Arg Val Ile Thr Glu Gln Val Lys
260 265 270

Glu Asn Met Lys Ala Val Leu Ala Asp Ile Gln Asn Gly Lys Phe Ala
275 280 285

Asn Asp Phe Val Asp Asp Tyr Lys Ala Gly Arg Pro Lys Leu Thr Ala
290 295 300

Tyr Arg Glu Gln Ala Ala Asn Leu Glu Ile Glu Lys Val Gly Ala Glu
305 310 315 320

Leu Arg Lys Ala Met Pro Phe Val Gly Lys Asn Asp Asp Asp Ala Phe
325 330 335

[0054]

Lys Ile Tyr Asn
340

<210> 53
<211> 1020
<212> DNA
<213> 唾液链球菌 (Streptococcus salivarius)

<400> 53
atggcagttc aaatggaata tgaaaaagac gtaaaagtag cagcacttga cggtaaaaaa 60

atcgccgtta tcggttacgg ttcacaaggt catgcacatg cacaaaactt gcgtgataca 120

ggtcacgatg tgattatcgg tgttcgtcca ggtaaatcat ttgataaagc taaagaagat 180

ggttttgaca cttacacagt agcagaagca actaaattgg ctgatgttat catgattttg 240

gctccagacg aaatccaaca agaactttac gaagcagaaa ttgctccaaa ccttgaagct 300

ggtaatgctg ttggttttgc acacggattt aacatccact tcgaattcat caaagttcct 360

aaagatgttg atgtcttcat gtgtgctcct aaaggaccag gtcacttggt acgtcgtaca 420

tttgaagaag gatttggtgt tcctgcactt tatgcagtat accaagatgc tacaggtaac 480

gctaaagata tcgctatgga ctggtgtaaa ggtgttggtg cagcacgtgt tggtcttctt 540

gaaacaacat acaaagaaga aactgaagaa gatttgttcg gtgaacaagc agttctttgt 600

ggtggtttga ctgcccttat cgaagctggt tttgaagtct tgactgaagc tggatatgct 660

ccagaattgg cttactttga agtgcttcac gaaatgaaat tgatcgttga cttgatttac 720

gaaggtggat tcaagaaaat gcgtcaatca atttctaaca ctgctgaatt cggtgactac 780

gtatcaggtc cacgtgttat cactgaacaa gtgaaagaaa acatgaaagc agttcttgct 840

gacatccaaa acggtaaatt cgctaacgac ttcgttaacg actacaaagc tggacgtcca 900

aaacttactg cttaccgtga agaagctgct aaccttgaaa tcgaaaaagt tggtgctgaa 960

ttgcgtaaag caatgccatt cgttggtcaa aacgacgatg acgcattcaa aatctataac 1020

<210> 54
<211> 340
<212> PRT
<213> 唾液链球菌 (Streptococcus salivarius)

<400> 54

Met Ala Val Gln Met Glu Tyr Glu Lys Asp Val Lys Val Ala Ala Leu
1 5 10 15

Asp Gly Lys Lys Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

His Ala Gln Asn Leu Arg Asp Thr Gly His Asp Val Ile Ile Gly Val
35 40 45

[0055]

Arg Pro Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Asp Thr
50 55 60

Tyr Thr Val Ala Glu Ala Thr Lys Leu Ala Asp Val Ile Met Ile Leu
65 70 75 80

Ala Pro Asp Glu Ile Gln Gln Glu Leu Tyr Glu Ala Glu Ile Ala Pro
85 90 95

Asn Leu Glu Ala Gly Asn Ala Val Gly Phe Ala His Gly Phe Asn Ile
100 105 110

His Phe Glu Phe Ile Lys Val Pro Lys Asp Val Asp Val Phe Met Cys
115 120 125

Ala Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Glu Glu Gly
130 135 140

Phe Gly Val Pro Ala Leu Tyr Ala Val Tyr Gln Asp Ala Thr Gly Asn
145 150 155 160

Ala Lys Asp Ile Ala Met Asp Trp Cys Lys Gly Val Gly Ala Ala Arg
165 170 175

Val Gly Leu Leu Glu Thr Thr Tyr Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Ile Glu
195 200 205

Ala Gly Phe Glu Val Leu Thr Glu Ala Gly Tyr Ala Pro Glu Leu Ala
210 215 220

Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Ile Tyr
225 230 235 240

Glu Gly Gly Phe Lys Lys Met Arg Gln Ser Ile Ser Asn Thr Ala Glu
245 250 255

Phe Gly Asp Tyr Val Ser Gly Pro Arg Val Ile Thr Glu Gln Val Lys
260 265 270

Glu Asn Met Lys Ala Val Leu Ala Asp Ile Gln Asn Gly Lys Phe Ala
275 280 285

Asn Asp Phe Val Asn Asp Tyr Lys Ala Gly Arg Pro Lys Leu Thr Ala
290 295 300

Tyr Arg Glu Glu Ala Ala Asn Leu Glu Ile Glu Lys Val Gly Ala Glu
305 310 315 320

[0056]

Leu Arg Lys Ala Met Pro Phe Val Gly Gln Asn Asp Asp Asp Ala Phe
325 330 335

Lys Ile Tyr Asn
340

<210> 55
<211> 1020
<212> DNA
<213> 嗜热链球菌 (Streptococcus thermophilus)

<400> 55
atggcagttc aaatggaata tgaaaaagac gtaaaagtac cagcacttga tggtaaaaaa 60

atcgccgtta tcggttacgg ttcacaaggt cacgctcact cacaaaactt gcgtgataca 120

ggtcacgatg ttatcatcgg tgttcgtcca ggtaaatcat ttgataaagc taaagaagat 180

ggttttgata cttacacagt agcagaagca actaaattgg ctgatgttat catgattttg 240

gctccagacg aaatccaaca agaactttac gaagcagaaa tcgctccaaa tcttgaagct 300

ggtaacgctg ttggttttgc tcacggtttc aacatccact ttgaatttat taaagtacca 360

gcagatgttg atgtatttat gtgtgcacct aaaggaccag gtcacttggt acgtcgtaca 420

tttgaagaag gttttggtgt acctgccctt tacgcagtat accaagatgc cacaggtaac 480

gctaaagaca tcgctatgga ctggtgtaaa ggtattggtg cagcacgtgt aggtcttctt 540

gaaacaacat acaaagaaga aactgaagaa gatttgtttg gtgaacaagc agttctttgt 600

ggtggtttga ctgcccttat cgaaactggt tttgaagtct tgactgaagc tggatatgca 660

ccagaattgg cttactttga agtacttcac gaaatgaaat taatcgttga cttgatttac 720

gaaggtggat tcaagaaaat gcgtcaatca atttctaaca ctgctgaatt cggtgactat 780

gtatcaggtc cacgtgttat cactgaacaa gttaaagaaa acatgaaagc agttcttgct 840

gatatccaaa atggtaaatt cgctaatgac ttcgttaacg actacaaagc tggacgtcca 900

aaacttactg cttaccgtga agaagctgct aaccttgaaa tcgaaaaagt tggtgctgaa 960

ttgcgtaaag caatgccatt cgttggtcaa aacgacgatg atgcattcaa aatctataac 1020

<210> 56
<211> 340
<212> PRT
<213> 嗜热链球菌 (Streptococcus thermophilus)

<400> 56

Met Ala Val Gln Met Glu Tyr Glu Lys Asp Val Lys Val Pro Ala Leu
1 5 10 15

Asp Gly Lys Lys Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

[0057]

His Ser Gln Asn Leu Arg Asp Thr Gly His Asp Val Ile Ile Gly Val
35 40 45

Arg Pro Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Asp Thr
50 55 60

Tyr Thr Val Ala Glu Ala Thr Lys Leu Ala Asp Val Ile Met Ile Leu
65 70 75 80

Ala Pro Asp Glu Ile Gln Gln Glu Leu Tyr Glu Ala Glu Ile Ala Pro
85 90 95

Asn Leu Glu Ala Gly Asn Ala Val Gly Phe Ala His Gly Phe Asn Ile
100 105 110

His Phe Glu Phe Ile Lys Val Pro Ala Asp Val Asp Val Phe Met Cys
115 120 125

Ala Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Glu Glu Gly
130 135 140

Phe Gly Val Pro Ala Leu Tyr Ala Val Tyr Gln Asp Ala Thr Gly Asn
145 150 155 160

Ala Lys Asp Ile Ala Met Asp Trp Cys Lys Gly Ile Gly Ala Ala Arg
165 170 175

Val Gly Leu Leu Glu Thr Thr Tyr Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Ile Glu
195 200 205

Thr Gly Phe Glu Val Leu Thr Glu Ala Gly Tyr Ala Pro Glu Leu Ala
210 215 220

Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Ile Tyr
225 230 235 240

Glu Gly Gly Phe Lys Lys Met Arg Gln Ser Ile Ser Asn Thr Ala Glu
245 250 255

Phe Gly Asp Tyr Val Ser Gly Pro Arg Val Ile Thr Glu Gln Val Lys
260 265 270

Glu Asn Met Lys Ala Val Leu Ala Asp Ile Gln Asn Gly Lys Phe Ala
275 280 285

Asn Asp Phe Val Asn Asp Tyr Lys Ala Gly Arg Pro Lys Leu Thr Ala
290 295 300

[0058]

Tyr Arg Glu Glu Ala Ala Asn Leu Glu Ile Glu Lys Val Gly Ala Glu
305 310 315 320

Leu Arg Lys Ala Met Pro Phe Val Gly Gln Asn Asp Asp Asp Ala Phe
325 330 335

Lys Ile Tyr Asn
340

<210> 57
<211> 975
<212> DNA
<213> 肺炎链球菌（Streptococcus pneumoniae）

<400> 57
ttgacggtaa aaaaatacgc cgttatcggt tatggttcac aagggcatgc gcatgctcaa 60

aacttgcgtg attcaggtcg tgacgttatt ataggtgtac gtccaggtaa atcttttgat 120

aaagcaaaag aagatggatt tgatacttac acagtagcag aagctactaa gttggctgat 180

gttatcatga tcttggcgcc agacgaaatt caacaagaat tgtacgaagc agaaatcgct 240

ccaaacttgg aagctggaaa cgcagttgga tttgcccatg gtttcaacat ccactttgaa 300

tttatcaaag ttcctgcgga tgtagatgtc ttcatgtgtg ctcctaaagg accaggacac 360

ttggtacgtc gtacttacga agaaggattt ggtgttccag ctctttatgc agtataccaa 420

gatgcaacag gaaatgctaa aaacattgct atggactggt gtaaaggtgt tggagcggct 480

cgtgtaggtc ttcttgaaac aacttacaaa gaagaaactg aagaagattt gtttggtgaa 540

caagctgtac tttgtggtgg tttgactgcc cttatcgaag caggtttcga agtcttgaca 600

gaagcaggtt acgctccaga attggcttac tttgaagttc ttcacgaaat gaaattgatc 660

gttgacttga tctacgaagg tggattcaag aaaatgcgtc aatctatttc aaacactgct 720

gaatacggtg actatgtatc aggtccacgt gtaatcactg aacaagttaa agaaaatatg 780

aaggctgtct tggcagacat ccaaaatggt aaatttgcaa atgactttgt aaatgactat 840

aaagctggac gtccaaaatt gactgcttac cgtgaacaag cagctaacct tgaaattgaa 900

aaagttggtg cagaattgcg taaagcaatg ccattcgttg gtaaaaacga cgatgatgca 960

ttcaaaatct ataac 975

<210> 58
<211> 325
<212> PRT
<213> 肺炎链球菌（Streptococcus pneumoniae）

<400> 58

Met Thr Val Lys Lys Tyr Ala Val Ile Gly Tyr Gly Ser Gln Gly His
1 5 10 15

[0059]

Ala His Ala Gln Asn Leu Arg Asp Ser Gly Arg Asp Val Ile Ile Gly
20 25 30

Val Arg Pro Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Asp
35 40 45

Thr Tyr Thr Val Ala Glu Ala Thr Lys Leu Ala Asp Val Ile Met Ile
50 55 60

Leu Ala Pro Asp Glu Ile Gln Gln Glu Leu Tyr Glu Ala Glu Ile Ala
65 70 75 80

Pro Asn Leu Glu Ala Gly Asn Ala Val Gly Phe Ala His Gly Phe Asn
85 90 95

Ile His Phe Glu Phe Ile Lys Val Pro Ala Asp Val Asp Val Phe Met
100 105 110

Cys Ala Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Tyr Glu Glu
115 120 125

Gly Phe Gly Val Pro Ala Leu Tyr Ala Val Tyr Gln Asp Ala Thr Gly
130 135 140

Asn Ala Lys Asn Ile Ala Met Asp Trp Cys Lys Gly Val Gly Ala Ala
145 150 155 160

Arg Val Gly Leu Leu Glu Thr Thr Tyr Lys Glu Glu Thr Glu Glu Asp
165 170 175

Leu Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Ile
180 185 190

Glu Ala Gly Phe Glu Val Leu Thr Glu Ala Gly Tyr Ala Pro Glu Leu
195 200 205

Ala Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Ile
210 215 220

Tyr Glu Gly Gly Phe Lys Lys Met Arg Gln Ser Ile Ser Asn Thr Ala
225 230 235 240

Glu Tyr Gly Asp Tyr Val Ser Gly Pro Arg Val Ile Thr Glu Gln Val
245 250 255

Lys Glu Asn Met Lys Ala Val Leu Ala Asp Ile Gln Asn Gly Lys Phe
260 265 270

Ala Asn Asp Phe Val Asn Asp Tyr Lys Ala Gly Arg Pro Lys Leu Thr
275 280 285

[0060]

Ala Tyr Arg Glu Gln Ala Ala Asn Leu Glu Ile Glu Lys Val Gly Ala
290 295 300

Glu Leu Arg Lys Ala Met Pro Phe Val Gly Lys Asn Asp Asp Asp Ala
305 310 315 320

Phe Lys Ile Tyr Asn
325

<210> 59
<211> 1020
<212> DNA
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 59
atggcagtta caatgtatta tgaagatgat gtagaagtat cagcacttgc tggaaagcaa 60

attgcagtaa tcggttatgg ttcacaagga catgctcacg cacagaattt gcgtgattct 120

ggtcacaacg ttatcattgg tgtgcgccac ggaaaatctt ttgataaagc aaaagaagat 180

ggctttgaaa catttgaagt aggagaagcg gtagctaaag ctgatgttat tatggttttg 240

gcgccagatg aacttcaaca atccatttat gaagaggaca tcaaaccaaa cttgaaagca 300

ggttcagcac ttggttttgc tcacggattt aatatccatt ttggctatat taaagtacca 360

gaagacgttg acgtctttat ggttgcacct aaggctccag gtcaccttgt ccgtcggact 420

tatactgaag gttttggtac accagctttg tttgtttcac accaaaatgc aagtggtcat 480

gcgcgtgaaa tcgcaatgga ttgggccaaa ggaattggtt gtgctcgagt gggaattatt 540

gaaacaacct ttaaagaaga aacagaagaa gatttgtttg gagaacaagc tgttctatgt 600

ggaggtttga cagcacttgt tgaagccggt tttgaaacac tgacagaagc tggatacgct 660

ggcgaattgg cttactttga agttttgcac gaaatgaaat tgattgttga cctcatgtat 720

gaaggtggtt ttactaaaat gcgtcaatcc atctcaaata ctgctgagtt tggcgattat 780

gtgactggtc caaggattat tactgacgca gttaaaaaga atatgaagct tgttttggct 840

gatattcaat ctggaaaatt tgctcaagat ttcgttgatg acttcaaagc ggggcgtcca 900

aaattaacag cctatcgcga agctgctaaa aatcttgaaa ttgaaaaaat tggggcagaa 960

ttacgtaaag caatgccatt cacacaatct ggtgatgacg atgcctttaa aatctatcag 1020

<210> 60
<211> 340
<212> PRT
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 60

Met Ala Val Thr Met Tyr Tyr Glu Asp Asp Val Glu Val Ser Ala Leu
1 5 10 15

[0061]

Ala Gly Lys Gln Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

His Ala Gln Asn Leu Arg Asp Ser Gly His Asn Val Ile Ile Gly Val
35 40 45

Arg His Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Glu Thr
50 55 60

Phe Glu Val Gly Glu Ala Val Ala Lys Ala Asp Val Ile Met Val Leu
65 70 75 80

Ala Pro Asp Glu Leu Gln Gln Ser Ile Tyr Glu Glu Asp Ile Lys Pro
85 90 95

Asn Leu Lys Ala Gly Ser Ala Leu Gly Phe Ala His Gly Phe Asn Ile
100 105 110

His Phe Gly Tyr Ile Lys Val Pro Glu Asp Val Asp Val Phe Met Val
115 120 125

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

Phe Gly Thr Pro Ala Leu Phe Val Ser His Gln Asn Ala Ser Gly His
145 150 155 160

Ala Arg Glu Ile Ala Met Asp Trp Ala Lys Gly Ile Gly Cys Ala Arg
165 170 175

Val Gly Ile Ile Glu Thr Thr Phe Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Val Glu
195 200 205

Ala Gly Phe Glu Thr Leu Thr Glu Ala Gly Tyr Ala Gly Glu Leu Ala
210 215 220

Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr
225 230 235 240

Glu Gly Gly Phe Thr Lys Met Arg Gln Ser Ile Ser Asn Thr Ala Glu
245 250 255

Phe Gly Asp Tyr Val Thr Gly Pro Arg Ile Ile Thr Asp Ala Val Lys
260 265 270

Lys Asn Met Lys Leu Val Leu Ala Asp Ile Gln Ser Gly Lys Phe Ala
275 280 285

[0062]

Gln Asp Phe Val Asp Asp Phe Lys Ala Gly Arg Pro Lys Leu Thr Ala
290 295 300

Tyr Arg Glu Ala Ala Lys Asn Leu Glu Ile Glu Lys Ile Gly Ala Glu
305 310 315 320

Leu Arg Lys Ala Met Pro Phe Thr Gln Ser Gly Asp Asp Asp Ala Phe
325 330 335

Lys Ile Tyr Gln
340

<210> 61
<211> 1041
<212> DNA
<213> 人葡萄球菌 (Staphylococcus hominis)

<400> 61
atgactacaa aaatgtttta tgacaaagat attgatacaa aaccattgga aaacaaaaag 60

attgcagtta ttggctatgg tgcacaaggg catgctcaag cgaataatct tcgcgactca 120

ggatttgatg tcatcatggg attgcgccca ggaaaatatt ttgatagtgc taaaaaagat 180

gggttcgaag tttactcagc tgctgaagca acggcacaag cggattttgt tatgatggaa 240

acccctgacg aattacaagc ggcagtttgg gagaaagaag ttgagcctaa ccttaaggca 300

ggttcttaca ttggattttc tcatgggttc aacattgttt atggtttgat taagcccaat 360

gctgatatta atgtcatgat catcgcgcca aagggtccag gaaacattga acgtcgccaa 420

ttcgttgaag gggttgggat tccttctttg tatggtgttc atcaagatcc tacgggtgat 480

acagctgaag tggccaaggc ctatgcgaaa ggtattggtt caggtcgcgc aggtatcttg 540

gaaacgactt ttgaagaaga aacaacagaa aacttgtttg gtgaacaggc tgtactttgt 600

ggtggcttaa cacaattgat tgaggcagga tttaacacat tggtggaagc aggttactca 660

cctgaattgg cttatttcga aacatctcat gaaatgaaga tgattgttga tttgatcttt 720

gaaggtggtt tcgagaagat gagacatgat tgctcaaata cttgtgaata tggtgaaatg 780

ttgaacggac cacgtatcat cactgaagaa tcaaagcaag gaatgcgtga tgttttgaag 840

gatatccaag atggtactta tgctaagaag tggttggcag aatacaattc tggtttgaag 900

gatttggaaa agatgcggac agagtataag agcggtttgt acgagcaaac aggtaagaag 960

gttcgtgcta tgatgccatg gatttcagat gcagataagt actcgacagc agcagatact 1020

gagcaattct cagcagcaaa g 1041

<210> 62
<211> 347
<212> PRT
<213> 人葡萄球菌 (Staphylococcus hominis)

[0063]

<400> 62

Met Thr Thr Lys Met Phe Tyr Asp Lys Asp Ile Asp Thr Lys Pro Leu
1 5 10 15

Glu Asn Lys Lys Ile Ala Val Ile Gly Tyr Gly Ala Gln Gly His Ala
20 25 30

Gln Ala Asn Asn Leu Arg Asp Ser Gly Phe Asp Val Ile Met Gly Leu
35 40 45

Arg Pro Gly Lys Tyr Phe Asp Ser Ala Lys Lys Asp Gly Phe Glu Val
50 55 60

Tyr Ser Ala Ala Glu Ala Thr Ala Gln Ala Asp Phe Val Met Met Glu
65 70 75 80

Thr Pro Asp Glu Leu Gln Ala Ala Val Trp Glu Lys Glu Val Glu Pro
85 90 95

Asn Leu Lys Ala Gly Ser Tyr Ile Gly Phe Ser His Gly Phe Asn Ile
100 105 110

Val Tyr Gly Leu Ile Lys Pro Asn Ala Asp Ile Asn Val Met Ile Ile
115 120 125

Ala Pro Lys Gly Pro Gly Asn Ile Glu Arg Arg Gln Phe Val Glu Gly
130 135 140

Val Gly Ile Pro Ser Leu Tyr Gly Val His Gln Asp Pro Thr Gly Asp
145 150 155 160

Thr Ala Glu Val Ala Lys Ala Tyr Ala Lys Gly Ile Gly Ser Gly Arg
165 170 175

Ala Gly Ile Leu Glu Thr Thr Phe Glu Glu Glu Thr Thr Glu Asn Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Gln Leu Ile Glu
195 200 205

Ala Gly Phe Asn Thr Leu Val Glu Ala Gly Tyr Ser Pro Glu Leu Ala
210 215 220

Tyr Phe Glu Thr Ser His Glu Met Lys Met Ile Val Asp Leu Ile Phe
225 230 235 240

Glu Gly Gly Phe Glu Lys Met Arg His Asp Cys Ser Asn Thr Cys Glu
245 250 255

[0064]

Tyr Gly Glu Met Leu Asn Gly Pro Arg Ile Ile Thr Glu Glu Ser Lys
260 265 270

Gln Gly Met Arg Asp Val Leu Lys Asp Ile Gln Asp Gly Thr Tyr Ala
275 280 285

Lys Lys Trp Leu Ala Glu Tyr Asn Ser Gly Leu Lys Asp Leu Glu Lys
290 295 300

Met Arg Thr Glu Tyr Lys Ser Gly Leu Tyr Glu Gln Thr Gly Lys Lys
305 310 315 320

Val Arg Ala Met Met Pro Trp Ile Ser Asp Ala Asp Lys Tyr Ser Thr
325 330 335

Ala Ala Asp Thr Glu Gln Phe Ser Ala Ala Lys
340 345

<210> 63
<211> 1041
<212> DNA
<213> 人葡萄球菌（Staphylococcus hominis）

<400> 63
atgactacaa aaatgtttta tgacaaagat attgatacaa aaccattgga aaacaaaaag 60

attgcagtta ttggctatgg tgcacaaggg catgctcaag cgaataatct tcgcgtctca 120

ggatttgatg tcatcatggg attgcgccca ggaaaatctt ttgatagtgt taaaaaagat 180

gggttcgaag tttactcagc tgctgaagca acggcacaag cggatgttgt tatgatggaa 240

acccctgacg aattacaagc ggcagtttgg gagaaagaag ttgagcctaa ccttaaggca 300

ggttctgacc ttggattttc tcatgggttc aacattgttt atggtttgat taagcccaat 360

gctgatatta atgtcatgat cattgcgcca aagggtccag gaaacattga acgtcgccaa 420

ttcgttgaag ggggtgggat tccttctttg tatggtgttc atcaagatcc tacgggtgat 480

acagctgaag tggccaaggc ctatgcgaaa ggtattggtt caggttgcgc aggtatcttg 540

gaaacgactt ttgaagaaga aacaacagaa gacttgtttg gtgaacaagc tgtactttgt 600

ggtggcttaa cacaattgat tgaggcagga tttaacacat tggtggaagc aggttactca 660

cctgaattgg cttatttcga aacatctcat gaaatgaaga tgattgttga tttgatcttt 720

gaaggtggtt tcgagaagat gagacatgat tgctcaaata cttgtgaata tggtgaaatg 780

ttgaacggac cacgtatcat cactgaagaa tcaaagcaag gaatgcgtga tgttttgaag 840

gatatccaag atggtactta tgctaagaag tggttggcag aatacaattc tggtttgaag 900

gatttggaaa agatgcggac agagtataag agcggtttgt acgagcaaac aggtaagaag 960

gttcgtgcta tgatgccatg gatttcagat gcagataagt actcgacagc agcagatact 1020

gagcaattct cagcagcaaa g 1041

[0065]

<210> 64
<211> 347
<212> PRT
<213> 人葡萄球菌 (Staphylococcus hominis)

<400> 64

Met Thr Thr Lys Met Phe Tyr Asp Lys Asp Ile Asp Thr Lys Pro Leu
1 5 10 15

Glu Asn Lys Lys Ile Ala Val Ile Gly Tyr Gly Ala Gln Gly His Ala
20 25 30

Gln Ala Asn Asn Leu Arg Val Ser Gly Phe Asp Val Ile Met Gly Leu
35 40 45

Arg Pro Gly Lys Ser Phe Asp Ser Val Lys Lys Asp Gly Phe Glu Val
50 55 60

Tyr Ser Ala Ala Glu Ala Thr Ala Gln Ala Asp Val Val Met Met Glu
65 70 75 80

Thr Pro Asp Glu Leu Gln Ala Ala Val Trp Glu Lys Glu Val Glu Pro
85 90 95

Asn Leu Lys Ala Gly Ser Asp Leu Gly Phe Ser His Gly Phe Asn Ile
100 105 110

Val Tyr Gly Leu Ile Lys Pro Asn Ala Asp Ile Asn Val Met Ile Ile
115 120 125

Ala Pro Lys Gly Pro Gly Asn Ile Glu Arg Arg Gln Phe Val Glu Gly
130 135 140

Gly Gly Ile Pro Ser Leu Tyr Gly Val His Gln Asp Pro Thr Gly Asp
145 150 155 160

Thr Ala Glu Val Ala Lys Ala Tyr Ala Lys Gly Ile Gly Ser Gly Cys
165 170 175

Ala Gly Ile Leu Glu Thr Thr Phe Glu Glu Glu Thr Thr Glu Asp Leu
180 185 190

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Gln Leu Ile Glu
195 200 205

Ala Gly Phe Asn Thr Leu Val Glu Ala Gly Tyr Ser Pro Glu Leu Ala
210 215 220

Tyr Phe Glu Thr Ser His Glu Met Lys Met Ile Val Asp Leu Ile Phe
225 230 235 240

[0066]

Glu Gly Gly Phe Glu Lys Met Arg His Asp Cys Ser Asn Thr Cys Glu
245 250 255

Tyr Gly Glu Met Leu Asn Gly Pro Arg Ile Ile Thr Glu Glu Ser Lys
260 265 270

Gln Gly Met Arg Asp Val Leu Lys Asp Ile Gln Asp Gly Thr Tyr Ala
275 280 285

Lys Lys Trp Leu Ala Glu Tyr Asn Ser Gly Leu Lys Asp Leu Glu Lys
290 295 300

Met Arg Thr Glu Tyr Lys Ser Gly Leu Tyr Glu Gln Thr Gly Lys Lys
305 310 315 320

Val Arg Ala Met Met Pro Trp Ile Ser Asp Ala Asp Lys Tyr Ser Thr
325 330 335

Ala Ala Asp Thr Glu Gln Phe Ser Ala Ala Lys
340 345

<210> 65
<211> 1038
<212> DNA
<213> 短乳杆菌 (Lactococcus brevis)

<400> 65
atgagtgtag aaatgttgta tgacaaggat gttaccacca ttatcttca aggaaagaaa 60

attgctttta tcggttatgg atctcaaggc catgcccaag ctaacaactt aagagattca 120

ggttatgacg ttatcgttgg tgttcgccca ggacaatcat ttgaaaatgc caaaatcgat 180

ggattcgatg tttacacacc agccgaagca gctcgtcgtg cagactggat tcaaatgtta 240

acgcctgatg aagttatggg tgatgtttat aaaaatgaaa ttgcacctaa ccttgaggaa 300

ggcaatgtat taggcttttc acatggcttc aacattcatt ataaagaaat cgtaccacca 360

gcaaacgttg atgttgttat gatggcacct aaaggcccag gtaatctttg tcgtcgtaca 420

tacgttgaag gctccggtgt tccagcatta tatggttatt tccaagacta ttcaggccat 480

gccgaagatt tatccaagga attcgccaaa ggaaatggtg cagcacgtgc cggactattg 540

aagacaacct tcaaagaaga aactgaagaa gatttgtttg gtgagcaaaa cgtccttatg 600

ggtggtgtta ccgcgctcat tgaaaccggt tatgaagtct taaccgaagc aggatactca 660

ccacagttgg catactttga agtagatcat gaaatgaaat tgatctgtga ccttatctac 720

gaaggtgggt tcaacaagat gtatgctgac tgctcgaaca cttctgaata tggttcatat 780

gtagttggtc caaaggtcgt tggtaaggaa tccaagcaag ctatgaaaga cgctttgaag 840

cggattcaag acggttcatt tgctaaagaa ttcatggatg actaccgtaa cggcttcaag 900

[0067]

aagctttaca agatgcgtga acgttcagct aactcacttc tttcacgtgt tggtgctgat 960

cttcgtgatc acatgtcatt tgttggtgaa gccgacaagt acagtacacc tactgaagaa 1020

aaagcagaag ctgaaaag 1038

<210> 66
<211> 346
<212> PRT
<213> 短乳杆菌 (Lactococcus brevis)

<400> 66

Met Ser Val Glu Met Leu Tyr Asp Lys Asp Val Thr Thr Asn Tyr Leu
1 5 10 15

Gln Gly Lys Lys Ile Ala Phe Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

Gln Ala Asn Asn Leu Arg Asp Ser Gly Tyr Asp Val Ile Val Gly Val
35 40 45

Arg Pro Gly Gln Ser Phe Glu Asn Ala Lys Ile Asp Gly Phe Asp Val
50 55 60

Tyr Thr Pro Ala Glu Ala Ala Arg Arg Ala Asp Trp Ile Gln Met Leu
65 70 75 80

Thr Pro Asp Glu Val Met Gly Asp Val Tyr Lys Asn Glu Ile Ala Pro
85 90 95

Asn Leu Glu Glu Gly Asn Val Leu Gly Phe Ser His Gly Phe Asn Ile
100 105 110

His Tyr Lys Glu Ile Val Pro Pro Ala Asn Val Asp Val Val Met Met
115 120 125

Ala Pro Lys Gly Pro Gly Asn Leu Cys Arg Arg Thr Tyr Val Glu Gly
130 135 140

Ser Gly Val Pro Ala Leu Tyr Gly Tyr Phe Gln Asp Tyr Ser Gly His
145 150 155 160

Ala Glu Asp Leu Ser Lys Glu Phe Ala Lys Gly Asn Gly Ala Ala Arg
165 170 175

Ala Gly Leu Leu Lys Thr Thr Phe Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

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

[0068]

Thr Gly Tyr Glu Val Leu Thr Glu Ala Gly Tyr Ser Pro Gln Leu Ala
210 215 220

Tyr Phe Glu Val Asp His Glu Met Lys Leu Ile Cys Asp Leu Ile Tyr
225 230 235 240

Glu Gly Gly Phe Asn Lys Met Tyr Ala Asp Cys Ser Asn Thr Ser Glu
245 250 255

Tyr Gly Ser Tyr Val Val Gly Pro Lys Val Val Gly Lys Glu Ser Lys
260 265 270

Gln Ala Met Lys Asp Ala Leu Lys Arg Ile Gln Asp Gly Ser Phe Ala
275 280 285

Lys Glu Phe Met Asp Asp Tyr Arg Asn Gly Phe Lys Lys Leu Tyr Lys
290 295 300

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

Leu Arg Asp His Met Ser Phe Val Gly Glu Ala Asp Lys Tyr Ser Thr
325 330 335

Pro Thr Glu Glu Lys Ala Glu Ala Glu Lys
340 345

<210> 67
<211> 1020
<212> DNA
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 67
atggcagtta caatgtatta tgaagatgat gtagaagtat cagcacttgc tggaaagcaa 60

attgcagtaa tcggttatgg ttcacaagga catgctcacg cacagaattt gcgtgattct 120

ggtcacaacg ttatcattgg tgtgcgccac ggaaaatctt ttgataaagc aaaagaagat 180

ggctttgaaa catttgaagt aggagaagca gtagctaaag ctgatgttat tatggttttg 240

gcaccagatg aacttcaaca atccatttat gaagaggaca tcaaaccaaa cttgaaagca 300

ggttcagcac ttggttttgc tcacggattt aatatccatt ttggctatat taaagtacca 360

gaagacgttg acgtctttat ggttgcgcct aaggctccag gtcaccttgt ccgtcggact 420

tatactgaag gttttggtac accagctttg tttgtttcac accaaaatgc aagtggtcat 480

gcgcgtgaaa tcgcaatgga ttgggccaaa ggaattggtt gtgctcgagt gggaattatt 540

gaaacaactt ttaaagaaga aacagaagaa gatttgtttg gagaacaagc tgttctatgt 600

ggaggtttga cagcacttgt tgaagccggt tttgaaacac tgacagaagc tggatacgct 660

ggcgaattgg cttactttga agttttgcac gaaatgaaat tgattgttga cctcatgtat 720

[0069]

gaaggtggtt ttactaaaat gcgtcaatcc atctcaaata ctgctgagtt tggcgattat 780

gtgactggtc cacggattat tactgacgaa gttaaaaaga atatgaagct tgttttggct 840

gatattcaat ctggaaaatt tgctcaagat ttcgttgatg acttcaaagc ggggcgtcca 900

aaattaatag cctatcgcga agctgcaaaa aatcttgaaa ttgaaaaaat tggggcagag 960

ctacgtcaag caatgccatt cacacaatct ggtgatgacg atgcctttaa aatctatcag 1020

<210> 68
<211> 340
<212> PRT
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 68

Met Ala Val Thr Met Tyr Tyr Glu Asp Asp Val Glu Val Ser Ala Leu
1 5 10 15

Ala Gly Lys Gln Ile Ala Val Ile Gly Tyr Gly Ser Gln Gly His Ala
20 25 30

His Ala Gln Asn Leu Arg Asp Ser Gly His Asn Val Ile Ile Gly Val
35 40 45

Arg His Gly Lys Ser Phe Asp Lys Ala Lys Glu Asp Gly Phe Glu Thr
50 55 60

Phe Glu Val Gly Glu Ala Val Ala Lys Ala Asp Val Ile Met Val Leu
65 70 75 80

Ala Pro Asp Glu Leu Gln Gln Ser Ile Tyr Glu Glu Asp Ile Lys Pro
85 90 95

Asn Leu Lys Ala Gly Ser Ala Leu Gly Phe Ala His Gly Phe Asn Ile
100 105 110

His Phe Gly Tyr Ile Lys Val Pro Glu Asp Val Asp Val Phe Met Val
115 120 125

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

Phe Gly Thr Pro Ala Leu Phe Val Ser His Gln Asn Ala Ser Gly His
145 150 155 160

Ala Arg Glu Ile Ala Met Asp Trp Ala Lys Gly Ile Gly Cys Ala Arg
165 170 175

Val Gly Ile Ile Glu Thr Thr Phe Lys Glu Glu Thr Glu Glu Asp Leu
180 185 190

[0070]

Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Leu Thr Ala Leu Val Glu
195 200 205

Ala Gly Phe Glu Thr Leu Thr Glu Ala Gly Tyr Ala Gly Glu Leu Ala
210 215 220

Tyr Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr
225 230 235 240

Glu Gly Gly Phe Thr Lys Met Arg Gln Ser Ile Ser Asn Thr Ala Glu
245 250 255

Phe Gly Asp Tyr Val Thr Gly Pro Arg Ile Ile Thr Asp Glu Val Lys
260 265 270

Lys Asn Met Lys Leu Val Leu Ala Asp Ile Gln Ser Gly Lys Phe Ala
275 280 285

Gln Asp Phe Val Asp Asp Phe Lys Ala Gly Arg Pro Lys Leu Ile Ala
290 295 300

Tyr Arg Glu Ala Ala Lys Asn Leu Glu Ile Glu Lys Ile Gly Ala Glu
305 310 315 320

Leu Arg Gln Ala Met Pro Phe Thr Gln Ser Gly Asp Asp Asp Ala Phe
325 330 335

Lys Ile Tyr Gln
340

<210> 69
<211> 1716
<212> DNA
<213> 枯草芽孢杆菌 (Bacillus subtilis)

<400> 69
atgttgacaa aagcaacaaa agaacaaaaa tcccttgtga aaaacagagg ggcggagctt 60

gttgttgatt gcttagtgga gcaaggtgtc acacatgtat ttggcattcc aggtgcaaaa 120

attgatgcgg tatttgacgc tttacaagat aaaggacctg aaattatcgt tgcccggcac 180

gaacaaaacg cagcattcat ggcccaagca gtcggccgtt taactggaaa accgggagtc 240

gtgttagtca catcaggacc gggtgcctct aacttggcaa caggcctgct gacagcgaac 300

actgaaggag accctgtcgt tgcgcttgct ggaaacgtga tccgtgcaga tcgtttaaaa 360

cggacacatc aatctttgga taatgcggcg ctattccagc cgattacaaa atacagtgta 420

gaagttcaag atgtaaaaaa tataccggaa gctgttacaa atgcatttag gatagcgtca 480

gcagggcagg ctggggccgc ttttgtgagc tttccgcaag atgttgtgaa tgaagtcaca 540

aatacgaaaa acgtgcgtgc tgttgcagcg ccaaaactcg gtcctgcagc agatgatgca 600

[0071]

atcagtgcgg ccatagcaaa aatccaaaca gcaaaacttc ctgtcgtttt ggtcggcatg 660

aaaggcggaa gaccggaagc aattaaagcg gttcgcaagc ttttgaaaaa ggttcagctt 720

ccatttgttg aaacatatca agctgccggt acccttttcta gagatttaga ggatcaatat 780

tttggccgta tcggtttgtt ccgcaaccag cctggcgatt tactgctaga gcaggcagat 840

gttgttctga cgatcggcta tgacccgatt gaatatgatc cgaaattctg gaatatcaat 900

ggagaccgga caattatcca tttagacgag attatcgctg acattgatca tgcttaccag 960

cctgatcttg aattgatcgg tgacattccg tccacgatca atcatatcga acacgatgct 1020

gtgaaagtgg aatttgcaga gcgtgagcag aaaatccttt ctgatttaaa acaatatatg 1080

catgaaggtg agcaggtgcc tgcagattgg aaatcagaca gagcgcaccc tcttgaaatc 1140

gttaaagagt tgcgtaatgc agtcgatgat catgttacag taacttgcga tatcggttcg 1200

cacgccattt ggatgtcacg ttatttccgc agctacgagc cgttaacatt aatgatcagt 1260

aacggtatgc aaacactcgg cgttgcgctt ccttgggcaa tcggcgcttc attggtgaaa 1320

ccgggagaaa aagtggtttc tgtctctggt gacggcggtt tcttattctc agcaatggaa 1380

ttagagacag cagttcgact aaaagcacca attgtacaca ttgtatggaa cgacagcaca 1440

tatgacatgg ttgcattcca gcaattgaaa aaatataacc gtacatctgc ggtcgatttc 1500

ggaaatatcg atatcgtgaa atatgcggaa agcttcggag caactggctt gcgcgtagaa 1560

tcaccagacc agctggcaga tgttctgcgt caaggcatga acgctgaagg tcctgtcatc 1620

atcgatgtcc cggttgacta cagtgataac attaatttag caagtgacaa gcttccgaaa 1680

gaattcgggg aactcatgaa aacgaaagct ctctag 1716

<210> 70
<211> 571
<212> PRT
<213> 枯草芽孢杆菌 (Bacillus subtilis)

<400> 70

Met Leu Thr Lys Ala Thr Lys Glu Gln Lys Ser Leu Val Lys Asn Arg
1 5 10 15

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

Val Phe Gly Ile Pro Gly Ala Lys Ile Asp Ala Val Phe Asp Ala Leu
35 40 45

Gln Asp Lys Gly Pro Glu Ile Ile Val Ala Arg His Glu Gln Asn Ala
50 55 60

Ala Phe Met Ala Gln Ala Val Gly Arg Leu Thr Gly Lys Pro Gly Val
65 70 75 80

[0072]

Val Leu Val Thr Ser Gly Pro Gly Ala Ser Asn Leu Ala Thr Gly Leu
85 90 95

Leu Thr Ala Asn Thr Glu Gly Asp Pro Val Val Ala Leu Ala Gly Asn
100 105 110

Val Ile Arg Ala Asp Arg Leu Lys Arg Thr His Gln Ser Leu Asp Asn
115 120 125

Ala Ala Leu Phe Gln Pro Ile Thr Lys Tyr Ser Val Glu Val Gln Asp
130 135 140

Val Lys Asn Ile Pro Glu Ala Val Thr Asn Ala Phe Arg Ile Ala Ser
145 150 155 160

Ala Gly Gln Ala Gly Ala Ala Phe Val Ser Phe Pro Gln Asp Val Val
165 170 175

Asn Glu Val Thr Asn Thr Lys Asn Val Arg Ala Val Ala Ala Pro Lys
180 185 190

Leu Gly Pro Ala Ala Asp Asp Ala Ile Ser Ala Ala Ile Ala Lys Ile
195 200 205

Gln Thr Ala Lys Leu Pro Val Val Leu Val Gly Met Lys Gly Gly Arg
210 215 220

Pro Glu Ala Ile Lys Ala Val Arg Lys Leu Leu Lys Lys Val Gln Leu
225 230 235 240

Pro Phe Val Glu Thr Tyr Gln Ala Ala Gly Thr Leu Ser Arg Asp Leu
245 250 255

Glu Asp Gln Tyr Phe Gly Arg Ile Gly Leu Phe Arg Asn Gln Pro Gly
260 265 270

Asp Leu Leu Leu Glu Gln Ala Asp Val Val Leu Thr Ile Gly Tyr Asp
275 280 285

Pro Ile Glu Tyr Asp Pro Lys Phe Trp Asn Ile Asn Gly Asp Arg Thr
290 295 300

Ile Ile His Leu Asp Glu Ile Ile Ala Asp Ile Asp His Ala Tyr Gln
305 310 315 320

Pro Asp Leu Glu Leu Ile Gly Asp Ile Pro Ser Thr Ile Asn His Ile
325 330 335

Glu His Asp Ala Val Lys Val Glu Phe Ala Glu Arg Glu Gln Lys Ile
340 345 350

[0073]

Leu Ser Asp Leu Lys Gln Tyr Met His Glu Gly Glu Gln Val Pro Ala
355 360 365

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

Arg Asn Ala Val Asp Asp His Val Thr Val Thr Cys Asp Ile Gly Ser
385 390 395 400

His Ala Ile Trp Met Ser Arg Tyr Phe Arg Ser Tyr Glu Pro Leu Thr
405 410 415

Leu Met Ile Ser Asn Gly Met Gln Thr Leu Gly Val Ala Leu Pro Trp
420 425 430

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

Ser Gly Asp Gly Gly Phe Leu Phe Ser Ala Met Glu Leu Glu Thr Ala
450 455 460

Val Arg Leu Lys Ala Pro Ile Val His Ile Val Trp Asn Asp Ser Thr
465 470 475 480

Tyr Asp Met Val Ala Phe Gln Gln Leu Lys Lys Tyr Asn Arg Thr Ser
485 490 495

Ala Val Asp Phe Gly Asn Ile Asp Ile Val Lys Tyr Ala Glu Ser Phe
500 505 510

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

Leu Arg Gln Gly Met Asn Ala Glu Gly Pro Val Ile Ile Asp Val Pro
530 535 540

Val Asp Tyr Ser Asp Asn Ile Asn Leu Ala Ser Asp Lys Leu Pro Lys
545 550 555 560

Glu Phe Gly Glu Leu Met Lys Thr Lys Ala Leu
565 570

<210> 71
<211> 1713
<212> DNA
<213> 人工序列

<220>
<223> 经密码子优化以在植物乳杆菌（Lactobacillus plantarum）中表达的编码区

[0074]

<400> 71
atgttgacca aggctaccaa agaacaaaag agtttagtca aaaaccgtgg tgctgaatta 60

gtcgtggatt gtttggttga acaaggtgtg acgcatgttt ttggtattcc aggagctaaa 120

attgatgccg tttttgatgc gttacaagat aagggtccag aaattattgt ggcacgtcat 180

gaacaaaatg cagcgtttat ggctcaagca gttggtcggt tgactggcaa accaggtgtg 240

gttttagtga cgtcaggtcc aggtgcgagt aatttagcga ctggcttgtt aacggcgaat 300

actgaaggtg atccagtcgt tgctttggca ggcaatgtca ttcgtgccga tcgtttaaag 360

cggacccatc agagtttgga taatgcagcc ttgtttcaac cgattacgaa atattcagtt 420

gaagtccaag atgtcaagaa tattccagaa gcggttacga atgcgtttcg tattgcatca 480

gctggccaag caggcgcagc gtttgtgagt tttccacaag atgtcgtgaa tgaagttact 540

aacaccaaga atgtccgtgc agtcgcagct ccaaagttag gtccagcagc tgacgatgcc 600

attagtgcag ctattgccaa aattcagact gcaaaattgc cggttgtgtt agttggcatg 660

aaaggtggtc gtccagaagc cattaaagcg gttcgtaagt tattgaaaaa ggttcaatta 720

ccatttgttg aaacgtatca agctgcaggt acgttaagtc gtgacttaga agatcaatat 780

tttggtcgga ttggtttgtt tcgtaatcaa ccaggtgatt tgttattaga acaagctgat 840

gtggttttaa ctattggcta tgatccgatt gaatatgatc caaagttttg gaatattaat 900

ggtgatcgta ccatcattca tttggatgaa atcattgctg atattgatca cgcttatcaa 960

ccggatttgg aattaattgg tgacattcca agtacgatta atcacattga acatgatgct 1020

gtgaaggttg agtttgcgga acgggaacag aaaattttat cagatttgaa gcaatatatg 1080

catgaaggtg aacaagtgcc agcagattgg aagtcagatc gggcccatcc attagaaatt 1140

gttaaagaat tacggaatgc agtggacgat catgtgaccg tgacttgtga tattggtagt 1200

catgctattt ggatgagtcg ttactttcgg tcatatgaac cgttaacttt aatgatttca 1260

aacggtatgc aaactttagg tgttgccttg ccatgggcca ttggtgcgtc attggtcaaa 1320

ccaggtgaaa aggtcgtgtc agtcagtgga gatggtggct tcttattcag tgctatggaa 1380

ttagaaaccg ctgtgcggtt gaaggcaccg attgtgcata ttgtgtggaa cgatagtact 1440

tatgatatgg tcgcatttca acagttgaag aaatataatc gtacctcagc agtggatttt 1500

ggtaatatcg atattgtcaa gtatgccgaa agttttggtg ccaccggttt gcgtgtcgaa 1560

tcaccagatc aattagctga tgtcttgcgt caaggtatga atgcggaagg cccagttatt 1620

attgatgtgc cagttgatta cagtgataac attaatttag ctagtgataa gttgccgaaa 1680

gaatttggtg aattaatgaa gacgaaagcg tta 1713

<210> 72
<211> 1680
<212> DNA
<213> 肺炎克雷伯氏菌（K. pneumonia）e

<400> 72

74

[0075]

atggacaaac agtatccggt acgccagtgg gcgcacggcg ccgatctcgt cgtcagtcag 60

ctggaagctc agggagtacg ccaggtgttc ggcatccccg gcgccaaaat cgacaaggtc 120

tttgattcac tgctggattc ctccattcgc attattccgg tacgccacga agccaacgcc 180

gcatttatgg ccgccgccgt cggacgcatt accggcaaag cgggcgtggc gctggtcacc 240

tccggtccgg gctgttccaa cctgatcacc ggcatggcca ccgcgaacag cgaaggcgac 300

ccggtggtgg ccctgggcgg cgcggtaaaa cgcgccgata aagcgaagca ggtccaccag 360

agtatggata cggtggcgat gttcagcccg gtcaccaaat acgccatcga ggtgacggcg 420

ccggatgcgc tggcggaagt ggtctccaac gccttccgcg ccgccgagca gggccggccg 480

ggcagcgcgt tcgttagcct gccgcaggat gtggtcgatg gcccggtcag cggcaaagtg 540

ctgccggcca gcggggcccc gcagatgggc gccgcgccgg atgatgccat cgaccaggtg 600

gcgaagctta tcgcccaggc gaagaacccg atcttcctgc tcggcctgat ggccagccag 660

ccggaaaaca gcaaggcgct gcgccgtttg ctggagacca gccatattcc agtcaccagc 720

acctatcagg ccgccggagc ggtgaatcag gataacttct ctcgcttcgc cggccgggtt 780

gggctgttta acaaccaggc cggggaccgt ctgctgcagc tcgccgacct ggtgatctgc 840

atcggctaca gcccggtgga atacgaaccg gcgatgtgga acagcggcaa cgcgacgctg 900

gtgcacatcg acgtgctgcc cgcctatgaa gagcgcaact acaccccgga tgtcgagctg 960

gtgggcgata tcgccggcac tctcaacaag ctggcgcaaa atatcgatca tcggctggtg 1020

ctctccccgc aggcggcgga tgcctccgc gaccgccagc accagcgcga gctgctggac 1080

cgccgcggcg cgcagctcaa ccagtttgcc ctgcatcccc tgcgcatcgt tcgcgccatg 1140

caggatatcg tcaacagcga cgtcacgttg accgtggaca tgggcagctt ccatatctgg 1200

attgcccgct acctgtacac gttccgcgcc cgtcaggtga tgatctccaa cggccagcag 1260

accatgggcg tcgccctgcc ctgggctatc ggcgcctggc tggtcaatcc tgagcgcaaa 1320

gtggtctccg tctccggcga cggcggcttc ctgcagtcga gcatggagct ggagaccgcc 1380

gtccgcctga aagccaacgt gctgcatctt atctgggtcg ataacggcta caacatggtc 1440

gctatccagg aagagaaaaa atatcagcgc ctgtccggcg tcgagtttgg gccgatggat 1500

tttaaagcct atgccgaatc cttcggcgcg aaagggtttg ccgtggaaag cgccgaggcg 1560

ctggagccga ccctgcgcgc ggcgatggac gtcgacggcc cggcggtagt ggccatcccg 1620

gtggattatc gcgataaccc gctgctgatg ggccagctgc atctgagtca gattctgtaa 1680

<210> 73
<211> 559
<212> PRT
<213> 肺炎克雷伯氏菌（K.pneumonia）e

<400> 73

Met Asp Lys Gln Tyr Pro Val Arg Gln Trp Ala His Gly Ala Asp Leu
1 5 10 15

[0076]

Val Val Ser Gln Leu Glu Ala Gln Gly Val Arg Gln Val Phe Gly Ile
20 25 30

Pro Gly Ala Lys Ile Asp Lys Val Phe Asp Ser Leu Leu Asp Ser Ser
35 40 45

Ile Arg Ile Ile Pro Val Arg His Glu Ala Asn Ala Ala Phe Met Ala
50 55 60

Ala Ala Val Gly Arg Ile Thr Gly Lys Ala Gly Val Ala Leu Val Thr
65 70 75 80

Ser Gly Pro Gly Cys Ser Asn Leu Ile Thr Gly Met Ala Thr Ala Asn
85 90 95

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

Asp Lys Ala Lys Gln Val His Gln Ser Met Asp Thr Val Ala Met Phe
115 120 125

Ser Pro Val Thr Lys Tyr Ala Ile Glu Val Thr Ala Pro Asp Ala Leu
130 135 140

Ala Glu Val Val Ser Asn Ala Phe Arg Ala Ala Glu Gln Gly Arg Pro
145 150 155 160

Gly Ser Ala Phe Val Ser Leu Pro Gln Asp Val Val Asp Gly Pro Val
165 170 175

Ser Gly Lys Val Leu Pro Ala Ser Gly Ala Pro Gln Met Gly Ala Ala
180 185 190

Pro Asp Asp Ala Ile Asp Gln Val Ala Lys Leu Ile Ala Gln Ala Lys
195 200 205

Asn Pro Ile Phe Leu Leu Gly Leu Met Ala Ser Gln Pro Glu Asn Ser
210 215 220

Lys Ala Leu Arg Arg Leu Leu Glu Thr Ser His Ile Pro Val Thr Ser
225 230 235 240

Thr Tyr Gln Ala Ala Gly Ala Val Asn Gln Asp Asn Phe Ser Arg Phe
245 250 255

Ala Gly Arg Val Gly Leu Phe Asn Asn Gln Ala Gly Asp Arg Leu Leu
260 265 270

[0077]

Gln Leu Ala Asp Leu Val Ile Cys Ile Gly Tyr Ser Pro Val Glu Tyr
275 280 285

Glu Pro Ala Met Trp Asn Ser Gly Asn Ala Thr Leu Val His Ile Asp
290 295 300

Val Leu Pro Ala Tyr Glu Glu Arg Asn Tyr Thr Pro Asp Val Glu Leu
305 310 315 320

Val Gly Asp Ile Ala Gly Thr Leu Asn Lys Leu Ala Gln Asn Ile Asp
325 330 335

His Arg Leu Val Leu Ser Pro Gln Ala Ala Glu Ile Leu Arg Asp Arg
340 345 350

Gln His Gln Arg Glu Leu Leu Asp Arg Arg Gly Ala Gln Leu Asn Gln
355 360 365

Phe Ala Leu His Pro Leu Arg Ile Val Arg Ala Met Gln Asp Ile Val
370 375 380

Asn Ser Asp Val Thr Leu Thr Val Asp Met Gly Ser Phe His Ile Trp
385 390 395 400

Ile Ala Arg Tyr Leu Tyr Thr Phe Arg Ala Arg Gln Val Met Ile Ser
405 410 415

Asn Gly Gln Gln Thr Met Gly Val Ala Leu Pro Trp Ala Ile Gly Ala
420 425 430

Trp Leu Val Asn Pro Glu Arg Lys Val Val Ser Val Ser Gly Asp Gly
435 440 445

Gly Phe Leu Gln Ser Ser Met Glu Leu Glu Thr Ala Val Arg Leu Lys
450 455 460

Ala Asn Val Leu His Leu Ile Trp Val Asp Asn Gly Tyr Asn Met Val
465 470 475 480

Ala Ile Gln Glu Glu Lys Lys Tyr Gln Arg Leu Ser Gly Val Glu Phe
485 490 495

Gly Pro Met Asp Phe Lys Ala Tyr Ala Glu Ser Phe Gly Ala Lys Gly
500 505 510

Phe Ala Val Glu Ser Ala Glu Ala Leu Glu Pro Thr Leu Arg Ala Ala
515 520 525

Met Asp Val Asp Gly Pro Ala Val Val Ala Ile Pro Val Asp Tyr Arg
530 535 540

[0078]

Asp Asn Pro Leu Leu Met Gly Gln Leu His Leu Ser Gln Ile Leu
545 550 555

<210> 74
<211> 1665
<212> DNA
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 74
atgtctgaga aacaatttgg ggcgaacttg gttgtcgata gtttgattaa ccataaagtg 60

aagtatgtat ttgggattcc aggagcaaaa attgaccggg tttttgattt attagaaaat 120

gaagaaggcc ctcaaatggt cgtgactcgt catgagcaag gagctgcttt catggctcaa 180

gctgtcggtc gtttaactgg cgaacctggt gtagtagttg ttacgagtgg gcctggtgta 240

tcaaaccttg cgactccgct tttgaccgcg acatcagaag gtgatgctat tttggctatc 300

ggtggacaag ttaaacgaag tgaccgtctt aaacgtgcgc accaatcaat ggataatgct 360

ggaatgatgc aatcagcaac aaaatattca gcagaagttc ttgaccctaa tacactttct 420

gaatcaattg ccaacgctta tcgtattgca aaatcaggac atccaggtgc aactttctta 480

tcaatccccc aagatgtaac ggatgccgaa gtatcaatca aagccattca accactttca 540

gaccctaaaa tggggaatgc ctctattgat gacattaatt atttagcaca agcaattaaa 600

aatgctgtat tgccagtaat tttggttgga gctggtgctt cagatgctaa agtcgcttca 660

tccttgcgta atctattgac tcatgttaat attcctgtcg ttgaaacatt ccaaggtgca 720

ggggttattt cacatgattt agaacatact ttttatggac gtatcggtct tttccgcaat 780

caaccaggcg atatgcttct gaaacgttct gaccttgtta ttgctgttgg ttatgaccca 840

attgaatatg aagctcgtaa ctggaatgca gaaattgata gtcgaattat cgttattgat 900

aatgccattg ctgaaattga tacttactac caaccagagc gtgaattaat tggtgatatc 960

gcagcaacat tggataatct tttaccagct gttcgtggct acaaaattcc aaaaggaaca 1020

aaagattatc tcgatggcct tcatgaagtt gctgagcaac acgaatttga tactgaaaat 1080

actgaagaag gtagaatgca ccctcttgat ttggtcagca ctttccaaga aatcgtcaag 1140

gatgatgaaa cagtaaccgt tgacgtaggt tcactctaca tttggatggc acgtcatttc 1200

aaatcatacg aaccacgtca tctcctcttc tcaaacggaa tgcaaacact cggagttgca 1260

cttccttggg caattacagc cgcattgttg cgcccaggta aaaaagttta ttcacactct 1320

ggtgatggag gcttcctttt cacagggcaa gaattggaaa cagctgtacg tttgaatctt 1380

ccaatcgttc aaattatctg gaatgacggc cattatgata tggttaaatt ccaagaagaa 1440

atgaaatatg gtcgttcagc agccgttgat tttggctatg ttgattacgt aaaatatgct 1500

gaagcaatga gagcaaaagg ttaccgtgca cacagcaaag aagaacttgc tgaaattctc 1560

aaatcaatcc cagatactac tggaccggtg gtaattgacg ttcctttgga ctattctgat 1620

[0079]

aacattaaat tagcagaaaa attattgcct gaagagtttt attga 1665

<210> 75
<211> 554
<212> PRT
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 75

Met Ser Glu Lys Gln Phe Gly Ala Asn Leu Val Val Asp Ser Leu Ile
1 5 10 15

Asn His Lys Val Lys Tyr Val Phe Gly Ile Pro Gly Ala Lys Ile Asp
20 25 30

Arg Val Phe Asp Leu Leu Glu Asn Glu Glu Gly Pro Gln Met Val Val
35 40 45

Thr Arg His Glu Gln Gly Ala Ala Phe Met Ala Gln Ala Val Gly Arg
50 55 60

Leu Thr Gly Glu Pro Gly Val Val Val Val Thr Ser Gly Pro Gly Val
65 70 75 80

Ser Asn Leu Ala Thr Pro Leu Leu Thr Ala Thr Ser Glu Gly Asp Ala
85 90 95

Ile Leu Ala Ile Gly Gly Gln Val Lys Arg Ser Asp Arg Leu Lys Arg
100 105 110

Ala His Gln Ser Met Asp Asn Ala Gly Met Met Gln Ser Ala Thr Lys
115 120 125

Tyr Ser Ala Glu Val Leu Asp Pro Asn Thr Leu Ser Glu Ser Ile Ala
130 135 140

Asn Ala Tyr Arg Ile Ala Lys Ser Gly His Pro Gly Ala Thr Phe Leu
145 150 155 160

Ser Ile Pro Gln Asp Val Thr Asp Ala Glu Val Ser Ile Lys Ala Ile
165 170 175

Gln Pro Leu Ser Asp Pro Lys Met Gly Asn Ala Ser Ile Asp Asp Ile
180 185 190

Asn Tyr Leu Ala Gln Ala Ile Lys Asn Ala Val Leu Pro Val Ile Leu
195 200 205

Val Gly Ala Gly Ala Ser Asp Ala Lys Val Ala Ser Ser Leu Arg Asn
210 215 220

[0080]

Leu Leu Thr His Val Asn Ile Pro Val Val Glu Thr Phe Gln Gly Ala
225 230 235 240

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

Leu Phe Arg Asn Gln Pro Gly Asp Met Leu Leu Lys Arg Ser Asp Leu
260 265 270

Val Ile Ala Val Gly Tyr Asp Pro Ile Glu Tyr Glu Ala Arg Asn Trp
275 280 285

Asn Ala Glu Ile Asp Ser Arg Ile Ile Val Ile Asp Asn Ala Ile Ala
290 295 300

Glu Ile Asp Thr Tyr Tyr Gln Pro Glu Arg Glu Leu Ile Gly Asp Ile
305 310 315 320

Ala Ala Thr Leu Asp Asn Leu Leu Pro Ala Val Arg Gly Tyr Lys Ile
325 330 335

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

Gln His Glu Phe Asp Thr Glu Asn Thr Glu Glu Gly Arg Met His Pro
355 360 365

Leu Asp Leu Val Ser Thr Phe Gln Glu Ile Val Lys Asp Asp Glu Thr
370 375 380

Val Thr Val Asp Val Gly Ser Leu Tyr Ile Trp Met Ala Arg His Phe
385 390 395 400

Lys Ser Tyr Glu Pro Arg His Leu Leu Phe Ser Asn Gly Met Gln Thr
405 410 415

Leu Gly Val Ala Leu Pro Trp Ala Ile Thr Ala Ala Leu Leu Arg Pro
420 425 430

Gly Lys Lys Val Tyr Ser His Ser Gly Asp Gly Gly Phe Leu Phe Thr
435 440 445

Gly Gln Glu Leu Glu Thr Ala Val Arg Leu Asn Leu Pro Ile Val Gln
450 455 460

Ile Ile Trp Asn Asp Gly His Tyr Asp Met Val Lys Phe Gln Glu Glu
465 470 475 480

Met Lys Tyr Gly Arg Ser Ala Ala Val Asp Phe Gly Tyr Val Asp Tyr
485 490 495

[0081]

Val Lys Tyr Ala Glu Ala Met Arg Ala Lys Gly Tyr Arg Ala His Ser
500 505 510

Lys Glu Glu Leu Ala Glu Ile Leu Lys Ser Ile Pro Asp Thr Thr Gly
515 520 525

Pro Val Val Ile Asp Val Pro Leu Asp Tyr Ser Asp Asn Ile Lys Leu
530 535 540

Ala Glu Lys Leu Leu Pro Glu Glu Phe Tyr
545 550

<210> 76
<211> 1665
<212> DNA
<213> 金黄色葡萄球菌（Staphylococcus aureus）

<220>
<221> CDS
<222> (1)..(1665)

<400> 76

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atg act gat aaa aag tac act gca gcc gat atg gtt att gat act ttg          48
Met Thr Asp Lys Lys Tyr Thr Ala Ala Asp Met Val Ile Asp Thr Leu
1               5                   10                  15

aaa aat aat ggg gta gaa tat gtt ttt ggt att ccg ggt gca aag ata          96
Lys Asn Asn Gly Val Glu Tyr Val Phe Gly Ile Pro Gly Ala Lys Ile
            20                  25                  30

gac tat cta ttt aat gct tta att gat gat ggt cct gaa ctt att gtc         144
Asp Tyr Leu Phe Asn Ala Leu Ile Asp Asp Gly Pro Glu Leu Ile Val
        35                  40                  45

act cgt cat gaa caa aat gct gca atg atg gca caa ggt att gga aga         192
Thr Arg His Glu Gln Asn Ala Ala Met Met Ala Gln Gly Ile Gly Arg
    50                  55                  60

tta aca ggt aaa ccg ggt gta gta ctt gtt aca agt ggc cct ggt gta         240
Leu Thr Gly Lys Pro Gly Val Val Leu Val Thr Ser Gly Pro Gly Val
65                  70                  75                  80

agt aat tta acg act gga cta tta aca gct aca tct gaa ggg gat cct         288
Ser Asn Leu Thr Thr Gly Leu Leu Thr Ala Thr Ser Glu Gly Asp Pro
                85                  90                  95

gta tta gcg tta ggt ggc caa gtg aaa cgt aat gat tta tta cga tta         336
Val Leu Ala Leu Gly Gly Gln Val Lys Arg Asn Asp Leu Leu Arg Leu
            100                 105                 110

acg cat caa agt att gat aat gct gcg cta tta aaa tat tca tca aaa         384
Thr His Gln Ser Ile Asp Asn Ala Ala Leu Leu Lys Tyr Ser Ser Lys
        115                 120                 125

tac agt gaa gaa gta caa gat cct gaa tca tta tca gaa gtt atg aca         432
Tyr Ser Glu Glu Val Gln Asp Pro Glu Ser Leu Ser Glu Val Met Thr
    130                 135                 140

aat gca att cga att gct act tca gga aaa aat ggc gca agt ttt att         480
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[0082]

```
Asn Ala Ile Arg Ile Ala Thr Ser Gly Lys Asn Gly Ala Ser Phe Ile
145                 150                 155                 160

agt att ccg caa gac gtt att tct tca cca gtt gaa tct aaa gct ata   528
Ser Ile Pro Gln Asp Val Ile Ser Ser Pro Val Glu Ser Lys Ala Ile
                165                 170                 175

tca ctt tgc caa aaa cca aat tta gga gta ccg agt gaa caa gat att   576
Ser Leu Cys Gln Lys Pro Asn Leu Gly Val Pro Ser Glu Gln Asp Ile
            180                 185                 190

aat gat gtc att gaa gcg att aaa aat gca tca ttt cct gtt tta tta   624
Asn Asp Val Ile Glu Ala Ile Lys Asn Ala Ser Phe Pro Val Leu Leu
        195                 200                 205

gct ggt atg aga agt tca agt gca gaa gaa aca aat gcc att cgc aaa   672
Ala Gly Met Arg Ser Ser Ser Ala Glu Glu Thr Asn Ala Ile Arg Lys
    210                 215                 220

tta gtt gag cgc acg aat tta cca gtt gta gaa aca ttc caa ggt gca   720
Leu Val Glu Arg Thr Asn Leu Pro Val Val Glu Thr Phe Gln Gly Ala
225                 230                 235                 240

ggt gta att agt cgt gaa tta gaa aat cat ttc ttc ggt cgt gtg ggc   768
Gly Val Ile Ser Arg Glu Leu Glu Asn His Phe Phe Gly Arg Val Gly
                245                 250                 255

tta ttc cgc aat caa gtt ggt gat gaa tta tta cgt aaa agt gat tta   816
Leu Phe Arg Asn Gln Val Gly Asp Glu Leu Leu Arg Lys Ser Asp Leu
            260                 265                 270

gtt gtt aca atc ggt tat gat cca att gaa tac gaa gct agt aac tgg   864
Val Val Thr Ile Gly Tyr Asp Pro Ile Glu Tyr Glu Ala Ser Asn Trp
        275                 280                 285

aat aaa gaa tta gaa aca caa att atc aat att gac gaa gtt caa gct   912
Asn Lys Glu Leu Glu Thr Gln Ile Ile Asn Ile Asp Glu Val Gln Ala
    290                 295                 300

gaa att act aat tat atg caa ccg aaa aaa gag ttg att ggt aat att   960
Glu Ile Thr Asn Tyr Met Gln Pro Lys Lys Glu Leu Ile Gly Asn Ile
305                 310                 315                 320

gct aaa acg att gaa atg att tct gaa aaa gtg gat gag cca ttt ata  1008
Ala Lys Thr Ile Glu Met Ile Ser Glu Lys Val Asp Glu Pro Phe Ile
                325                 330                 335

aat caa caa cat tta gac gaa tta gaa caa tta aga aca cat att gat  1056
Asn Gln Gln His Leu Asp Glu Leu Glu Gln Leu Arg Thr His Ile Asp
            340                 345                 350

gaa gaa act ggt att aaa gcg acg cat gaa gaa gga att cta cat cca  1104
Glu Glu Thr Gly Ile Lys Ala Thr His Glu Glu Gly Ile Leu His Pro
        355                 360                 365

gtg gaa att att gaa tct atg caa aag gta tta act gat gat act act  1152
Val Glu Ile Ile Glu Ser Met Gln Lys Val Leu Thr Asp Asp Thr Thr
    370                 375                 380

gta aca gtt gat gtt gga agt cac tat att tgg atg gca cgt aat ttc  1200
Val Thr Val Asp Val Gly Ser His Tyr Ile Trp Met Ala Arg Asn Phe
385                 390                 395                 400

aga agt tac aat cca aga cat tta tta ttt agc aat ggt atg caa acg  1248
Arg Ser Tyr Asn Pro Arg His Leu Leu Phe Ser Asn Gly Met Gln Thr
                405                 410                 415
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[0083]

```
ctt ggt gta gca tta ccg tgg gca att tca gct gca ctt gtg cgc cct      1296
Leu Gly Val Ala Leu Pro Trp Ala Ile Ser Ala Ala Leu Val Arg Pro
            420                 425                 430

aat acg caa gtt gtg tcc gtt gct ggc gat ggt ggc ttt tta ttt tca      1344
Asn Thr Gln Val Val Ser Val Ala Gly Asp Gly Gly Phe Leu Phe Ser
        435                 440                 445

tca caa gat tta gaa acg gcc gta cgt aaa aat tta aat atc atc cag      1392
Ser Gln Asp Leu Glu Thr Ala Val Arg Lys Asn Leu Asn Ile Ile Gln
    450                 455                 460

ctt att tgg aat gat gga aaa tat aac atg gtt gaa ttc caa gaa gaa      1440
Leu Ile Trp Asn Asp Gly Lys Tyr Asn Met Val Glu Phe Gln Glu Glu
465                 470                 475                 480

atg aaa tat aaa cgt tcg tca ggt gta gac ttc ggt cct gta gat ttt      1488
Met Lys Tyr Lys Arg Ser Ser Gly Val Asp Phe Gly Pro Val Asp Phe
                485                 490                 495

gta aaa tat gca gaa tca ttt ggc gcg aaa ggt tta cga gtt act aat      1536
Val Lys Tyr Ala Glu Ser Phe Gly Ala Lys Gly Leu Arg Val Thr Asn
            500                 505                 510

caa gaa gaa tta gaa gcg gca att aaa gag ggc tat gaa aca gat ggt      1584
Gln Glu Glu Leu Glu Ala Ala Ile Lys Glu Gly Tyr Glu Thr Asp Gly
        515                 520                 525

cca gta tta att gat ata cct gta aat tac aaa gat aat atc aaa ctt      1632
Pro Val Leu Ile Asp Ile Pro Val Asn Tyr Lys Asp Asn Ile Lys Leu
    530                 535                 540

tca aca aat atg tta cct gac gta ttt aac taa                         1665
Ser Thr Asn Met Leu Pro Asp Val Phe Asn
545                 550
```

<210> 77
<211> 554
<212> PRT
<213> 金黄色葡萄球菌（Staphylococcus aureus）

<400> 77

```
Met Thr Asp Lys Lys Tyr Thr Ala Ala Asp Met Val Ile Asp Thr Leu
1               5                   10                  15

Lys Asn Asn Gly Val Glu Tyr Val Phe Gly Ile Pro Gly Ala Lys Ile
            20                  25                  30

Asp Tyr Leu Phe Asn Ala Leu Ile Asp Asp Gly Pro Glu Leu Ile Val
        35                  40                  45

Thr Arg His Glu Gln Asn Ala Ala Met Met Ala Gln Gly Ile Gly Arg
    50                  55                  60

Leu Thr Gly Lys Pro Gly Val Val Leu Val Thr Ser Gly Pro Gly Val
65                  70                  75                  80

Ser Asn Leu Thr Thr Gly Leu Leu Thr Ala Thr Ser Glu Gly Asp Pro
```

[0084]

85 90 95

Val Leu Ala Leu Gly Gly Gln Val Lys Arg Asn Asp Leu Leu Arg Leu
100 105 110

Thr His Gln Ser Ile Asp Asn Ala Ala Leu Leu Lys Tyr Ser Ser Lys
115 120 125

Tyr Ser Glu Glu Val Gln Asp Pro Glu Ser Leu Ser Glu Val Met Thr
130 135 140

Asn Ala Ile Arg Ile Ala Thr Ser Gly Lys Asn Gly Ala Ser Phe Ile
145 150 155 160

Ser Ile Pro Gln Asp Val Ile Ser Ser Pro Val Glu Ser Lys Ala Ile
165 170 175

Ser Leu Cys Gln Lys Pro Asn Leu Gly Val Pro Ser Glu Gln Asp Ile
180 185 190

Asn Asp Val Ile Glu Ala Ile Lys Asn Ala Ser Phe Pro Val Leu Leu
195 200 205

Ala Gly Met Arg Ser Ser Ser Ala Glu Glu Thr Asn Ala Ile Arg Lys
210 215 220

Leu Val Glu Arg Thr Asn Leu Pro Val Val Glu Thr Phe Gln Gly Ala
225 230 235 240

Gly Val Ile Ser Arg Glu Leu Glu Asn His Phe Phe Gly Arg Val Gly
245 250 255

Leu Phe Arg Asn Gln Val Gly Asp Glu Leu Leu Arg Lys Ser Asp Leu
260 265 270

Val Val Thr Ile Gly Tyr Asp Pro Ile Glu Tyr Glu Ala Ser Asn Trp
275 280 285

Asn Lys Glu Leu Glu Thr Gln Ile Ile Asn Ile Asp Glu Val Gln Ala
290 295 300

Glu Ile Thr Asn Tyr Met Gln Pro Lys Lys Glu Leu Ile Gly Asn Ile
305 310 315 320

Ala Lys Thr Ile Glu Met Ile Ser Glu Lys Val Asp Glu Pro Phe Ile
325 330 335

Asn Gln Gln His Leu Asp Glu Leu Glu Gln Leu Arg Thr His Ile Asp
340 345 350

[0085]

Glu Glu Thr Gly Ile Lys Ala Thr His Glu Glu Gly Ile Leu His Pro
355 360 365

Val Glu Ile Ile Glu Ser Met Gln Lys Val Leu Thr Asp Asp Thr Thr
370 375 380

Val Thr Val Asp Val Gly Ser His Tyr Ile Trp Met Ala Arg Asn Phe
385 390 395 400

Arg Ser Tyr Asn Pro Arg His Leu Leu Phe Ser Asn Gly Met Gln Thr
405 410 415

Leu Gly Val Ala Leu Pro Trp Ala Ile Ser Ala Ala Leu Val Arg Pro
420 425 430

Asn Thr Gln Val Val Ser Val Ala Gly Asp Gly Gly Phe Leu Phe Ser
435 440 445

Ser Gln Asp Leu Glu Thr Ala Val Arg Lys Asn Leu Asn Ilc Ile Gln
450 455 460

Leu Ile Trp Asn Asp Gly Lys Tyr Asn Met Val Glu Phe Gln Glu Glu
465 470 475 480

Met Lys Tyr Lys Arg Ser Ser Gly Val Asp Phe Gly Pro Val Asp Phe
485 490 495

Val Lys Tyr Ala Glu Ser Phe Gly Ala Lys Gly Leu Arg Val Thr Asn
500 505 510

Gln Glu Glu Leu Glu Ala Ala Ilc Lys Glu Gly Tyr Glu Thr Asp Gly
515 520 525

Pro Val Leu Ile Asp Ile Pro Val Asn Tyr Lys Asp Asn Ile Lys Leu
530 535 540

Ser Thr Asn Met Leu Pro Asp Val Phe Asn
545 550

<210> 78
<211> 1698
<212> DNA
<213> 单核细胞增多性李斯特菌（Listeria monocytogenes）

<220>
<221> CDS
<222> (1)..(1698)

<400> 78
atg gcg aaa cta gaa aaa gac caa gaa aaa gta ata aca caa ggg aaa 48
Met Ala Lys Leu Glu Lys Asp Gln Glu Lys Val Ile Thr Gln Gly Lys

[0086]

```
1               5                   10                  15

tca gga gcg gat tta gtt gta gac agc tta att aat caa ggt gtt acg      96
Ser Gly Ala Asp Leu Val Val Asp Ser Leu Ile Asn Gln Gly Val Thr
            20                  25                  30

cat gta ttc ggg att ccg gga gcg aaa att gat aaa gtt ttt gat gtg     144
His Val Phe Gly Ile Pro Gly Ala Lys Ile Asp Lys Val Phe Asp Val
        35                  40                  45

atg gaa gaa cgt gga cca gaa tta att gtc agt cgt cat gaa caa aat     192
Met Glu Glu Arg Gly Pro Glu Leu Ile Val Ser Arg His Glu Gln Asn
    50                  55                  60

gcg gcg ttt atg gct gct gct atc ggt cgt cta acc ggg aaa cct ggt     240
Ala Ala Phe Met Ala Ala Ala Ile Gly Arg Leu Thr Gly Lys Pro Gly
65                  70                  75                  80

gtt gta ctt gta act agt gga cct ggc gca tcg aat ctt gca aca ggg     288
Val Val Leu Val Thr Ser Gly Pro Gly Ala Ser Asn Leu Ala Thr Gly
                85                  90                  95

ctt gta acc gca act gca gaa gga gat cca gtc gtt gcg att gct ggt     336
Leu Val Thr Ala Thr Ala Glu Gly Asp Pro Val Val Ala Ile Ala Gly
            100                 105                 110

aac gta aca agg caa gac cgc tta aaa aga acc cac caa tca atg gat     384
Asn Val Thr Arg Gln Asp Arg Leu Lys Arg Thr His Gln Ser Met Asp
        115                 120                 125

aat gca gca ctt ttc cgt ccg att aca aaa tac agc gaa gaa gta gtt     432
Asn Ala Ala Leu Phe Arg Pro Ile Thr Lys Tyr Ser Glu Glu Val Val
    130                 135                 140

cac gcc gaa agt att cca gaa gca atc act aac gct ttt cgc tcg gca     480
His Ala Glu Ser Ile Pro Glu Ala Ile Thr Asn Ala Phe Arg Ser Ala
145                 150                 155                 160

aca gaa cca aac caa ggc gct gct ttt gtc agt ttg cca caa gat atc     528
Thr Glu Pro Asn Gln Gly Ala Ala Phe Val Ser Leu Pro Gln Asp Ile
                165                 170                 175

gtg aac gaa cca aac gta cca gta aaa gcg att cgc cca ctt gct aaa     576
Val Asn Glu Pro Asn Val Pro Val Lys Ala Ile Arg Pro Leu Ala Lys
            180                 185                 190

cca gaa aat ggt cct gct tcc aaa gaa caa gtt gca aaa ctt gtt aca     624
Pro Glu Asn Gly Pro Ala Ser Lys Glu Gln Val Ala Lys Leu Val Thr
        195                 200                 205

cgt ttg aaa aaa gcg aaa tta ccg gta ttg cta ttg ggt atg cga gca     672
Arg Leu Lys Lys Ala Lys Leu Pro Val Leu Leu Leu Gly Met Arg Ala
    210                 215                 220

tct agt cca gaa gta act ggt gca att cgt cgc tta ctc caa aaa aca     720
Ser Ser Pro Glu Val Thr Gly Ala Ile Arg Arg Leu Leu Gln Lys Thr
225                 230                 235                 240

agt atc cca gta gta gaa act ttc caa gca gct ggc gtc att tca cgc     768
Ser Ile Pro Val Val Glu Thr Phe Gln Ala Ala Gly Val Ile Ser Arg
                245                 250                 255

gac tta gaa gat aac ttc ttt gga cgt gtt ggt ctg ttc cgc aac caa     816
Asp Leu Glu Asp Asn Phe Phe Gly Arg Val Gly Leu Phe Arg Asn Gln
            260                 265                 270
```

[0087]

```
cca ggg gat att ttg tta aat aaa gct gat tta gtt att aca gtg ggt      864
Pro Gly Asp Ile Leu Leu Asn Lys Ala Asp Leu Val Ile Thr Val Gly
        275                 280                 285

tat gat cca att gaa tac gat cca aaa gct tgg aat gcc tct ggt gat      912
Tyr Asp Pro Ile Glu Tyr Asp Pro Lys Ala Trp Asn Ala Ser Gly Asp
    290                 295                 300

aga acg att gtc cat tta gac gac att cgc gct gat att gat cat tat      960
Arg Thr Ile Val His Leu Asp Asp Ile Arg Ala Asp Ile Asp His Tyr
305                 310                 315                 320

tac caa cca gtg aca gag cta gtc gga aac atc gcg ctt act tta gac     1008
Tyr Gln Pro Val Thr Glu Leu Val Gly Asn Ile Ala Leu Thr Leu Asp
                325                 330                 335

cga gtg aat gcg aaa ttc agc ggt tta gaa tta gcg gaa aaa gaa ctt     1056
Arg Val Asn Ala Lys Phe Ser Gly Leu Glu Leu Ala Glu Lys Glu Leu
            340                 345                 350

gaa aca tta aaa gaa ctt cat gct caa tta gaa gag cga gat gtt ccg     1104
Glu Thr Leu Lys Glu Leu His Ala Gln Leu Glu Glu Arg Asp Val Pro
        355                 360                 365

cca gaa agt gat gaa act aac cga gta cat cca ttg tcg gtc att caa     1152
Pro Glu Ser Asp Glu Thr Asn Arg Val His Pro Leu Ser Val Ile Gln
    370                 375                 380

aca cta cgt tcg gca att gat gac aac gta act gtg aca gtc gac gtt     1200
Thr Leu Arg Ser Ala Ile Asp Asp Asn Val Thr Val Thr Val Asp Val
385                 390                 395                 400

ggt tca cat tat att tgg atg gca cgt cat ttc cgc tcc tat gaa cca     1248
Gly Ser His Tyr Ile Trp Met Ala Arg His Phe Arg Ser Tyr Glu Pro
                405                 410                 415

cgc cgt ctg ctt ttc agt aac ggt atg caa acg ctt ggt gtt gcg ctt     1296
Arg Arg Leu Leu Phe Ser Asn Gly Met Gln Thr Leu Gly Val Ala Leu
            420                 425                 430

cct tgg gga att gct gca aca ctt gta cat ccg ggt gaa aaa gtg gtt     1344
Pro Trp Gly Ile Ala Ala Thr Leu Val His Pro Gly Glu Lys Val Val
        435                 440                 445

tcg att tct ggt gac ggt ggt ttc tta ttt tcc gcg atg gaa tta gaa     1392
Ser Ile Ser Gly Asp Gly Gly Phe Leu Phe Ser Ala Met Glu Leu Glu
    450                 455                 460

aca gct gtc cgc ttg cgt gcg cca ctt gta cac cta gta tgg aat gac     1440
Thr Ala Val Arg Leu Arg Ala Pro Leu Val His Leu Val Trp Asn Asp
465                 470                 475                 480

gga agc tat gac atg gtt gct ttc caa caa aaa atg aaa tac ggc aaa     1488
Gly Ser Tyr Asp Met Val Ala Phe Gln Gln Lys Met Lys Tyr Gly Lys
                485                 490                 495

gaa gca gct gtt cgt ttt ggc gat gtt gat atc gta aaa ttt gca gaa     1536
Glu Ala Ala Val Arg Phe Gly Asp Val Asp Ile Val Lys Phe Ala Glu
            500                 505                 510

agt ttc gga gca aaa ggt ctt cgc gta aca aat cca gca gaa ctt tct     1584
Ser Phe Gly Ala Lys Gly Leu Arg Val Thr Asn Pro Ala Glu Leu Ser
        515                 520                 525

gat gtg tta aaa gaa gcg ctt gaa aca gaa gga ccc gtc gtt gta gat     1632
Asp Val Leu Lys Glu Ala Leu Glu Thr Glu Gly Pro Val Val Val Asp
```

[0088]

530 535 540

att cca att gat tac cgt gat aac atc aaa ctt ggc gaa act tta cta 1680
Ile Pro Ile Asp Tyr Arg Asp Asn Ile Lys Leu Gly Glu Thr Leu Leu
545 550 555 560

cct gac caa ttt tat taa 1698
Pro Asp Gln Phe Tyr
565

<210> 79
<211> 565
<212> PRT
<213> 单核细胞增多性李斯特菌（Listeria monocytogenes）

<400> 79

Met Ala Lys Leu Glu Lys Asp Gln Glu Lys Val Ile Thr Gln Gly Lys
1 5 10 15

Ser Gly Ala Asp Leu Val Val Asp Ser Leu Ile Asn Gln Gly Val Thr
20 25 30

His Val Phe Gly Ile Pro Gly Ala Lys Ile Asp Lys Val Phe Asp Val
35 40 45

Met Glu Glu Arg Gly Pro Glu Leu Ile Val Ser Arg His Glu Gln Asn
50 55 60

Ala Ala Phe Met Ala Ala Ala Ile Gly Arg Leu Thr Gly Lys Pro Gly
65 70 75 80

Val Val Leu Val Thr Ser Gly Pro Gly Ala Ser Asn Leu Ala Thr Gly
85 90 95

Leu Val Thr Ala Thr Ala Glu Gly Asp Pro Val Val Ala Ile Ala Gly
100 105 110

Asn Val Thr Arg Gln Asp Arg Leu Lys Arg Thr His Gln Ser Met Asp
115 120 125

Asn Ala Ala Leu Phe Arg Pro Ile Thr Lys Tyr Ser Glu Glu Val Val
130 135 140

His Ala Glu Ser Ile Pro Glu Ala Ile Thr Asn Ala Phe Arg Ser Ala
145 150 155 160

Thr Glu Pro Asn Gln Gly Ala Ala Phe Val Ser Leu Pro Gln Asp Ile
165 170 175

Val Asn Glu Pro Asn Val Pro Val Lys Ala Ile Arg Pro Leu Ala Lys
180 185 190

[0089]

Pro Glu Asn Gly Pro Ala Ser Lys Glu Gln Val Ala Lys Leu Val Thr
195 200 205

Arg Leu Lys Lys Ala Lys Leu Pro Val Leu Leu Leu Gly Met Arg Ala
210 215 220

Ser Ser Pro Glu Val Thr Gly Ala Ile Arg Arg Leu Leu Gln Lys Thr
225 230 235 240

Ser Ile Pro Val Val Glu Thr Phe Gln Ala Ala Gly Val Ile Ser Arg
245 250 255

Asp Leu Glu Asp Asn Phe Phe Gly Arg Val Gly Leu Phe Arg Asn Gln
260 265 270

Pro Gly Asp Ile Leu Leu Asn Lys Ala Asp Leu Val Ile Thr Val Gly
275 280 285

Tyr Asp Pro Ile Glu Tyr Asp Pro Lys Ala Trp Asn Ala Ser Gly Asp
290 295 300

Arg Thr Ile Val His Leu Asp Asp Ile Arg Ala Asp Ile Asp His Tyr
305 310 315 320

Tyr Gln Pro Val Thr Glu Leu Val Gly Asn Ile Ala Leu Thr Leu Asp
325 330 335

Arg Val Asn Ala Lys Phe Ser Gly Leu Glu Leu Ala Glu Lys Glu Leu
340 345 350

Glu Thr Leu Lys Glu Leu His Ala Gln Leu Glu Glu Arg Asp Val Pro
355 360 365

Pro Glu Ser Asp Glu Thr Asn Arg Val His Pro Leu Ser Val Ile Gln
370 375 380

Thr Leu Arg Ser Ala Ile Asp Asp Asn Val Thr Val Thr Val Asp Val
385 390 395 400

Gly Ser His Tyr Ile Trp Met Ala Arg His Phe Arg Ser Tyr Glu Pro
405 410 415

Arg Arg Leu Leu Phe Ser Asn Gly Met Gln Thr Leu Gly Val Ala Leu
420 425 430

Pro Trp Gly Ile Ala Ala Thr Leu Val His Pro Gly Glu Lys Val Val
435 440 445

Ser Ile Ser Gly Asp Gly Gly Phe Leu Phe Ser Ala Met Glu Leu Glu
450 455 460

[0090]

Thr Ala Val Arg Leu Arg Ala Pro Leu Val His Leu Val Trp Asn Asp
465 470 475 480

Gly Ser Tyr Asp Met Val Ala Phe Gln Gln Lys Met Lys Tyr Gly Lys
485 490 495

Glu Ala Ala Val Arg Phe Gly Asp Val Asp Ile Val Lys Phe Ala Glu
500 505 510

Ser Phe Gly Ala Lys Gly Leu Arg Val Thr Asn Pro Ala Glu Leu Ser
515 520 525

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

Ile Pro Ile Asp Tyr Arg Asp Asn Ile Lys Leu Gly Glu Thr Leu Leu
545 550 555 560

Pro Asp Gln Phe Tyr
565

<210> 80
<211> 1680
<212> DNA
<213> 变异链球菌（Streptococcus mutans）

<220>
<221> CDS
<222> (1)..(1680)

<400> 80
atg acc gaa ata aat aag gaa ggc tat ggg gct gac ctg att gta gac 48
Met Thr Glu Ile Asn Lys Glu Gly Tyr Gly Ala Asp Leu Ile Val Asp
1 5 10 15

agc ctc att aat cat gat gtc aac tat gtt ttt gga atc cct ggt gca 96
Ser Leu Ile Asn His Asp Val Asn Tyr Val Phe Gly Ile Pro Gly Ala
20 25 30

aaa att gat cgt gtc ttt gat acc tta gaa gat aag ggg cca gaa ctt 144
Lys Ile Asp Arg Val Phe Asp Thr Leu Glu Asp Lys Gly Pro Glu Leu
35 40 45

att gta gca cgc cat gag caa aat gct gct ttt atg gct caa gga att 192
Ile Val Ala Arg His Glu Gln Asn Ala Ala Phe Met Ala Gln Gly Ile
50 55 60

ggc cgt att act ggt gag cct ggt gtt gtg att aca acc agc ggt ccc 240
Gly Arg Ile Thr Gly Glu Pro Gly Val Val Ile Thr Thr Ser Gly Pro
65 70 75 80

ggt gtt tcc aat ctg gtg act ggt ctt gtt act gcg aca gct gag gga 288
Gly Val Ser Asn Leu Val Thr Gly Leu Val Thr Ala Thr Ala Glu Gly
85 90 95

gat cct gtc ctt gct att ggt ggt cag gtt aaa cgt gct gat ttg ctc 336

[0091]

```
Asp Pro Val Leu Ala Ile Gly Gly Gln Val Lys Arg Ala Asp Leu Leu
            100                 105                 110

aaa cgg gct cac cag tca atg aat aat gtt gct atg ctc gat ccc att      384
Lys Arg Ala His Gln Ser Met Asn Asn Val Ala Met Leu Asp Pro Ile
        115                 120                 125

acc aaa tat tca gca gaa att cag gat ccc gca aca ctt tca gaa aat      432
Thr Lys Tyr Ser Ala Glu Ile Gln Asp Pro Ala Thr Leu Ser Glu Asn
    130                 135                 140

att gct aat gcc tat cgt ttg gct aaa gca gga aag ccg gga gct agt      480
Ile Ala Asn Ala Tyr Arg Leu Ala Lys Ala Gly Lys Pro Gly Ala Ser
145                 150                 155                 160

ttc tta tct att cct caa gat ata act gat agt cct gtt act gtc aag      528
Phe Leu Ser Ile Pro Gln Asp Ile Thr Asp Ser Pro Val Thr Val Lys
                165                 170                 175

gcg att aag ccc ttg aca gat cct aaa cta ggt tca gcg tca gtt gct      576
Ala Ile Lys Pro Leu Thr Asp Pro Lys Leu Gly Ser Ala Ser Val Ala
            180                 185                 190

gat att aat tat ttg gca cag gcc ata aaa aat gcg gtc ctt cct gtc      624
Asp Ile Asn Tyr Leu Ala Gln Ala Ile Lys Asn Ala Val Leu Pro Val
        195                 200                 205

tta ctt tta gga aat ggt gcg tca acg gct gca gtt aca gct tct att      672
Leu Leu Leu Gly Asn Gly Ala Ser Thr Ala Ala Val Thr Ala Ser Ile
    210                 215                 220

cgc cgt ttg tta gga gct gtc aag ctg cca gtc gtt gaa act ttc caa      720
Arg Arg Leu Leu Gly Ala Val Lys Leu Pro Val Val Glu Thr Phe Gln
225                 230                 235                 240

gga gct ggt att gtt tca aga gat tta gaa gag gac act ttt ttt ggt      768
Gly Ala Gly Ile Val Ser Arg Asp Leu Glu Glu Asp Thr Phe Phe Gly
                245                 250                 255

cgt gtg ggg ctt ttt cgt aat cag ccc gga gat atg ttg ctg aag cgt      816
Arg Val Gly Leu Phe Arg Asn Gln Pro Gly Asp Met Leu Leu Lys Arg
            260                 265                 270

tct gac tta gtt atc gct att ggc tat gat cct att gaa tat gaa gcg      864
Ser Asp Leu Val Ile Ala Ile Gly Tyr Asp Pro Ile Glu Tyr Glu Ala
        275                 280                 285

cgc aat tgg aat gct gaa att tcg gct cgc att atc gtt att gat gtt      912
Arg Asn Trp Asn Ala Glu Ile Ser Ala Arg Ile Ile Val Ile Asp Val
    290                 295                 300

gct cca gct gaa att gat act tat ttc caa cct gaa cgt gaa tta att      960
Ala Pro Ala Glu Ile Asp Thr Tyr Phe Gln Pro Glu Arg Glu Leu Ile
305                 310                 315                 320

ggt gat ata gct gaa aca ctt gat tta ctc cta cct gct att agt ggc     1008
Gly Asp Ile Ala Glu Thr Leu Asp Leu Leu Leu Pro Ala Ile Ser Gly
                325                 330                 335

tac tca ctt cca aaa ggt tct ctt gac tat ctc aaa ggc ctt cgt gat     1056
Tyr Ser Leu Pro Lys Gly Ser Leu Asp Tyr Leu Lys Gly Leu Arg Asp
            340                 345                 350

aat gta gta gaa gat gtc aaa ttt gat aag aca gtc aaa tcc ggt ctg     1104
Asn Val Val Glu Asp Val Lys Phe Asp Lys Thr Val Lys Ser Gly Leu
        355                 360                 365
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[0092]

```
gtt cat ccg ctt gat gtg att gat gtc ctt caa aag caa acg act gat      1152
Val His Pro Leu Asp Val Ile Asp Val Leu Gln Lys Gln Thr Thr Asp
    370                 375                 380

gat atg aca gta acg gtt gat gtt ggc agc cat tat att tgg atg gct      1200
Asp Met Thr Val Thr Val Asp Val Gly Ser His Tyr Ile Trp Met Ala
385                 390                 395                 400

cgt tat ttt aaa agc tat gaa gca cgg cac tta ctt ttc tca aat ggt      1248
Arg Tyr Phe Lys Ser Tyr Glu Ala Arg His Leu Leu Phe Ser Asn Gly
                405                 410                 415

atg caa acc tta ggt gtt gct ttg cct tgg gca att tcg gca gct ctt      1296
Met Gln Thr Leu Gly Val Ala Leu Pro Trp Ala Ile Ser Ala Ala Leu
            420                 425                 430

gta cgg cca aat gag aag att att tct att tca ggt gat ggt ggt ttc      1344
Val Arg Pro Asn Glu Lys Ile Ile Ser Ile Ser Gly Asp Gly Gly Phe
        435                 440                 445

ctc ttt tct ggc caa gaa ttg gaa aca gct gtt cgt tta cat tta cca      1392
Leu Phe Ser Gly Gln Glu Leu Glu Thr Ala Val Arg Leu His Leu Pro
    450                 455                 460

att gtt cat atc att tgg aat gat ggt aaa tat aat atg gtt gaa ttc      1440
Ile Val His Ile Ile Trp Asn Asp Gly Lys Tyr Asn Met Val Glu Phe
465                 470                 475                 480

caa gaa gaa atg aaa tac ggc cgt tca gca ggt gtt gat ttt ggt cct      1488
Gln Glu Glu Met Lys Tyr Gly Arg Ser Ala Gly Val Asp Phe Gly Pro
                485                 490                 495

gtt gat ttt gtc aag tat gct gat agt ttc ggt gct aaa ggt tac cgt      1536
Val Asp Phe Val Lys Tyr Ala Asp Ser Phe Gly Ala Lys Gly Tyr Arg
            500                 505                 510

gct gat agt aaa gaa aag ttt gat caa gtt ctt caa aca gca ctc aag      1584
Ala Asp Ser Lys Glu Lys Phe Asp Gln Val Leu Gln Thr Ala Leu Lys
        515                 520                 525

gaa gct gca aat ggc cca gtt ctc att gat gtt cca atg gac tat aaa      1632
Glu Ala Ala Asn Gly Pro Val Leu Ile Asp Val Pro Met Asp Tyr Lys
    530                 535                 540

gat aat gta aaa ttg ggt gaa act att ttg cct gat gaa ttc tac taa      1680
Asp Asn Val Lys Leu Gly Glu Thr Ile Leu Pro Asp Glu Phe Tyr
545                 550                 555
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<210> 81
<211> 559
<212> PRT
<213> 变异链球菌（Streptococcus mutans）

<400> 81

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Met Thr Glu Ile Asn Lys Glu Gly Tyr Gly Ala Asp Leu Ile Val Asp
1               5                   10                  15

Ser Leu Ile Asn His Asp Val Asn Tyr Val Phe Gly Ile Pro Gly Ala
            20                  25                  30

Lys Ile Asp Arg Val Phe Asp Thr Leu Glu Asp Lys Gly Pro Glu Leu
```

[0093]

35 40 45

Ile Val Ala Arg His Glu Gln Asn Ala Ala Phe Met Ala Gln Gly Ile
50 55 60

Gly Arg Ile Thr Gly Glu Pro Gly Val Val Ile Thr Thr Ser Gly Pro
65 70 75 80

Gly Val Ser Asn Leu Val Thr Gly Leu Val Thr Ala Thr Ala Glu Gly
85 90 95

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

Lys Arg Ala His Gln Ser Met Asn Asn Val Ala Met Leu Asp Pro Ile
115 120 125

Thr Lys Tyr Ser Ala Glu Ile Gln Asp Pro Ala Thr Leu Ser Glu Asn
130 135 140

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

Phe Leu Ser Ile Pro Gln Asp Ile Thr Asp Ser Pro Val Thr Val Lys
165 170 175

Ala Ile Lys Pro Leu Thr Asp Pro Lys Leu Gly Ser Ala Ser Val Ala
180 185 190

Asp Ile Asn Tyr Leu Ala Gln Ala Ile Lys Asn Ala Val Leu Pro Val
195 200 205

Leu Leu Leu Gly Asn Gly Ala Ser Thr Ala Ala Val Thr Ala Ser Ile
210 215 220

Arg Arg Leu Leu Gly Ala Val Lys Leu Pro Val Val Glu Thr Phe Gln
225 230 235 240

Gly Ala Gly Ile Val Ser Arg Asp Leu Glu Glu Asp Thr Phe Phe Gly
245 250 255

Arg Val Gly Leu Phe Arg Asn Gln Pro Gly Asp Met Leu Leu Lys Arg
260 265 270

Ser Asp Leu Val Ile Ala Ile Gly Tyr Asp Pro Ile Glu Tyr Glu Ala
275 280 285

Arg Asn Trp Asn Ala Glu Ile Ser Ala Arg Ile Ile Val Ile Asp Val
290 295 300

[0094]

Ala Pro Ala Glu Ile Asp Thr Tyr Phe Gln Pro Glu Arg Glu Leu Ile
305 310 315 320

Gly Asp Ile Ala Glu Thr Leu Asp Leu Leu Leu Pro Ala Ile Ser Gly
325 330 335

Tyr Ser Leu Pro Lys Gly Ser Leu Asp Tyr Leu Lys Gly Leu Arg Asp
340 345 350

Asn Val Val Glu Asp Val Lys Phe Asp Lys Thr Val Lys Ser Gly Leu
355 360 365

Val His Pro Leu Asp Val Ile Asp Val Leu Gln Lys Gln Thr Thr Asp
370 375 380

Asp Met Thr Val Thr Val Asp Val Gly Ser His Tyr Ile Trp Met Ala
385 390 395 400

Arg Tyr Phe Lys Ser Tyr Glu Ala Arg His Leu Leu Phe Ser Asn Gly
405 410 415

Met Gln Thr Leu Gly Val Ala Leu Pro Trp Ala Ile Ser Ala Ala Leu
420 425 430

Val Arg Pro Asn Glu Lys Ile Ile Ser Ile Ser Gly Asp Gly Gly Phe
435 440 445

Leu Phe Ser Gly Gln Glu Leu Glu Thr Ala Val Arg Leu His Leu Pro
450 455 460

Ile Val His Ile Ile Trp Asn Asp Gly Lys Tyr Asn Met Val Glu Phe
465 470 475 480

Gln Glu Glu Met Lys Tyr Gly Arg Ser Ala Gly Val Asp Phe Gly Pro
485 490 495

Val Asp Phe Val Lys Tyr Ala Asp Ser Phe Gly Ala Lys Gly Tyr Arg
500 505 510

Ala Asp Ser Lys Glu Lys Phe Asp Gln Val Leu Gln Thr Ala Leu Lys
515 520 525

Glu Ala Ala Asn Gly Pro Val Leu Ile Asp Val Pro Met Asp Tyr Lys
530 535 540

Asp Asn Val Lys Leu Gly Glu Thr Ile Leu Pro Asp Glu Phe Tyr
545 550 555

<210> 82

[0095]

<211> 1683
<212> DNA
<213> 嗜热链球菌 (Streptococcus thermophilus)

<220>
<221> CDS
<222> (1)..(1683)

<400> 82

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gtg ttc atg tca gaa gaa aag caa ttg tat ggt gca gat tta gtg gtt          48
Val Phe Met Ser Glu Glu Lys Gln Leu Tyr Gly Ala Asp Leu Val Val
1               5                  10                  15

gat agt ttg atc aac cat gat gtt gag tat gtc ttt ggg att cca ggc          96
Asp Ser Leu Ile Asn His Asp Val Glu Tyr Val Phe Gly Ile Pro Gly
            20                  25                  30

gca aaa atc gat agg gtt ttt gat acc ttg gaa gat aag gga cct gaa         144
Ala Lys Ile Asp Arg Val Phe Asp Thr Leu Glu Asp Lys Gly Pro Glu
        35                  40                  45

ttg att gtt gcc cgt cat gag caa aat gct gct ttt atg gct caa ggt         192
Leu Ile Val Ala Arg His Glu Gln Asn Ala Ala Phe Met Ala Gln Gly
    50                  55                  60

gtt gga cgt att act ggg aaa cca ggt gta gta ttg gta aca tct ggt         240
Val Gly Arg Ile Thr Gly Lys Pro Gly Val Val Leu Val Thr Ser Gly
65                  70                  75                  80

cca ggt gtc tcc aat ttg gct act ggt ttg gta aca gcg acg gat gaa         288
Pro Gly Val Ser Asn Leu Ala Thr Gly Leu Val Thr Ala Thr Asp Glu
                85                  90                  95

gga gac cct gtt ctt gct att ggt ggt cag gtt aag cgt gca gat ctc         336
Gly Asp Pro Val Leu Ala Ile Gly Gly Gln Val Lys Arg Ala Asp Leu
            100                 105                 110

ttg aaa cgt gcc cac caa tca atg aat aac gtt gct atg ctt gag cca         384
Leu Lys Arg Ala His Gln Ser Met Asn Asn Val Ala Met Leu Glu Pro
        115                 120                 125

att acc aaa tat gct gct gaa gta cat gat gct aac acc ctt tct gaa         432
Ile Thr Lys Tyr Ala Ala Glu Val His Asp Ala Asn Thr Leu Ser Glu
    130                 135                 140

acg gtt gct aat gcc tat cgt cac gct aag tca ggg aaa cca ggt gca         480
Thr Val Ala Asn Ala Tyr Arg His Ala Lys Ser Gly Lys Pro Gly Ala
145                 150                 155                 160

agc ttc att tca att cct caa gac gtg acg gat gct ccg gtc agt gtt         528
Ser Phe Ile Ser Ile Pro Gln Asp Val Thr Asp Ala Pro Val Ser Val
                165                 170                 175

aag gct att aag cct atg aca gat cca aaa ctt ggt tca gca tct gtt         576
Lys Ala Ile Lys Pro Met Thr Asp Pro Lys Leu Gly Ser Ala Ser Val
            180                 185                 190

tct gat att aac tat cta gca caa gcc att aaa aat gca gtg ttg cca         624
Ser Asp Ile Asn Tyr Leu Ala Gln Ala Ile Lys Asn Ala Val Leu Pro
        195                 200                 205

gtc ttt ctt ttg ggg aat ggt gcc tca tca gaa gcc gta act tac tct         672
Val Phe Leu Leu Gly Asn Gly Ala Ser Ser Glu Ala Val Thr Tyr Ser
    210                 215                 220
```

[0096]

```
att cgc caa att ttg aag cat gtt aaa ttg cca gtt gtt gaa act ttc          720
Ile Arg Gln Ile Leu Lys His Val Lys Leu Pro Val Val Glu Thr Phe
225                 230                 235                 240

caa ggt gcc ggt atc gtg tca cgt gac ctt gaa gaa gat act ttc ttt          768
Gln Gly Ala Gly Ile Val Ser Arg Asp Leu Glu Glu Asp Thr Phe Phe
                245                 250                 255

ggt cgt gta ggt ctt ttc cgt aac caa ccc gga gac atg ttg ctt aaa          816
Gly Arg Val Gly Leu Phe Arg Asn Gln Pro Gly Asp Met Leu Leu Lys
            260                 265                 270

aaa tcc gac tta gtt att gcc att ggt tat gat cca atc gaa tat gaa          864
Lys Ser Asp Leu Val Ile Ala Ile Gly Tyr Asp Pro Ile Glu Tyr Glu
        275                 280                 285

gca cgt aac tgg aat gct gaa att tca gca cgt atc atc gtt att gat          912
Ala Arg Asn Trp Asn Ala Glu Ile Ser Ala Arg Ile Ile Val Ile Asp
    290                 295                 300

gtc gag ccg gcc gag gtg gac act tac ttc caa ccg gaa cgt gaa ttg          960
Val Glu Pro Ala Glu Val Asp Thr Tyr Phe Gln Pro Glu Arg Glu Leu
305                 310                 315                 320

att ggt aat gta gaa gcg agc tta gac ttg ctt ttg ccc gct att caa         1008
Ile Gly Asn Val Glu Ala Ser Leu Asp Leu Leu Leu Pro Ala Ile Gln
                325                 330                 335

ggt tat aaa ttg cct gaa ggt gcg gtt gaa tat ctt aaa ggt ttg aaa         1056
Gly Tyr Lys Leu Pro Glu Gly Ala Val Glu Tyr Leu Lys Gly Leu Lys
            340                 345                 350

aac aat gtt gtt gag gat gtt aag ttt gac cgt cag cct gat gaa ggt         1104
Asn Asn Val Val Glu Asp Val Lys Phe Asp Arg Gln Pro Asp Glu Gly
        355                 360                 365

acg gtg cat ccg cta gat ttc atc gaa aat ttg caa gaa cac aca gat         1152
Thr Val His Pro Leu Asp Phe Ile Glu Asn Leu Gln Glu His Thr Asp
    370                 375                 380

gat gat atg act gtt acg ttt gat gtt ggt agt cac tat att tgg atg         1200
Asp Asp Met Thr Val Thr Phe Asp Val Gly Ser His Tyr Ile Trp Met
385                 390                 395                 400

gca cgt tat ctc aaa tcg tat gaa cca cgt cat ttg ctt ttc tca aat         1248
Ala Arg Tyr Leu Lys Ser Tyr Glu Pro Arg His Leu Leu Phe Ser Asn
                405                 410                 415

ggg atg caa acg ata ggt att gct att aca tgg gct atc tct gca gca         1296
Gly Met Gln Thr Ile Gly Ile Ala Ile Thr Trp Ala Ile Ser Ala Ala
            420                 425                 430

ttg gtt cgt cct aag aca aaa gtg att tct gta tct ggt gat ggt ggt         1344
Leu Val Arg Pro Lys Thr Lys Val Ile Ser Val Ser Gly Asp Gly Gly
        435                 440                 445

ttc ctc ttc tca gca caa gaa ttg gaa aca gca gtt cgt ttg aaa ttg         1392
Phe Leu Phe Ser Ala Gln Glu Leu Glu Thr Ala Val Arg Leu Lys Leu
    450                 455                 460

cca att gtc cat att atc tgg aac gat ggt cat tac aat atg gtg gaa         1440
Pro Ile Val His Ile Ile Trp Asn Asp Gly His Tyr Asn Met Val Glu
465                 470                 475                 480

ttc cag gaa gaa atg aag tac ggt cgt tca tct ggg gtt gac ttt ggt         1488
Phe Gln Glu Glu Met Lys Tyr Gly Arg Ser Ser Gly Val Asp Phe Gly
```

[0097]

485 490 495

cct gta gat ttt gta aaa tat gct gag agc ttt gga gcc aaa ggt tat 1536
Pro Val Asp Phe Val Lys Tyr Ala Glu Ser Phe Gly Ala Lys Gly Tyr
500 505 510

cgt gca aca agt aaa gca gcg ttt gct agc ttg ctt caa gag gct ttg 1584
Arg Ala Thr Ser Lys Ala Ala Phe Ala Ser Leu Leu Gln Glu Ala Leu
515 520 525

act cag gct gta gat gga cca gtc ctt att gat gtt cca att gac tat 1632
Thr Gln Ala Val Asp Gly Pro Val Leu Ile Asp Val Pro Ile Asp Tyr
530 535 540

aaa gat aac att aaa ctc ggc gaa act att ttg cca gat gaa ttt tac 1680
Lys Asp Asn Ile Lys Leu Gly Glu Thr Ile Leu Pro Asp Glu Phe Tyr
545 550 555 560

taa 1683

<210> 83
<211> 560
<212> PRT
<213> 嗜热链球菌 (Streptococcus thermophilus)

<400> 83

Val Phe Met Ser Glu Glu Lys Gln Leu Tyr Gly Ala Asp Leu Val Val
1 5 10 15

Asp Ser Leu Ile Asn His Asp Val Glu Tyr Val Phe Gly Ile Pro Gly
20 25 30

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

Leu Ile Val Ala Arg His Glu Gln Asn Ala Ala Phe Met Ala Gln Gly
50 55 60

Val Gly Arg Ile Thr Gly Lys Pro Gly Val Val Leu Val Thr Ser Gly
65 70 75 80

Pro Gly Val Ser Asn Leu Ala Thr Gly Leu Val Thr Ala Thr Asp Glu
85 90 95

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

Leu Lys Arg Ala His Gln Ser Met Asn Asn Val Ala Met Leu Glu Pro
115 120 125

Ile Thr Lys Tyr Ala Ala Glu Val His Asp Ala Asn Thr Leu Ser Glu
130 135 140

Thr Val Ala Asn Ala Tyr Arg His Ala Lys Ser Gly Lys Pro Gly Ala
145 150 155 160

[0098]

Ser Phe Ile Ser Ile Pro Gln Asp Val Thr Asp Ala Pro Val Ser Val
165 170 175

Lys Ala Ile Lys Pro Met Thr Asp Pro Lys Leu Gly Ser Ala Ser Val
180 185 190

Ser Asp Ile Asn Tyr Leu Ala Gln Ala Ile Lys Asn Ala Val Leu Pro
195 200 205

Val Phe Leu Leu Gly Asn Gly Ala Ser Ser Glu Ala Val Thr Tyr Ser
210 215 220

Ile Arg Gln Ile Leu Lys His Val Lys Leu Pro Val Val Glu Thr Phe
225 230 235 240

Gln Gly Ala Gly Ile Val Ser Arg Asp Leu Glu Glu Asp Thr Phe Phe
245 250 255

Gly Arg Val Gly Leu Phe Arg Asn Gln Pro Gly Asp Met Leu Leu Lys
260 265 270

Lys Ser Asp Leu Val Ile Ala Ile Gly Tyr Asp Pro Ile Glu Tyr Glu
275 280 285

Ala Arg Asn Trp Asn Ala Glu Ile Ser Ala Arg Ile Ile Val Ile Asp
290 295 300

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

Ile Gly Asn Val Glu Ala Ser Leu Asp Leu Leu Leu Pro Ala Ile Gln
325 330 335

Gly Tyr Lys Leu Pro Glu Gly Ala Val Glu Tyr Leu Lys Gly Leu Lys
340 345 350

Asn Asn Val Val Glu Asp Val Lys Phe Asp Arg Gln Pro Asp Glu Gly
355 360 365

Thr Val His Pro Leu Asp Phe Ile Glu Asn Leu Gln Glu His Thr Asp
370 375 380

Asp Asp Met Thr Val Thr Phe Asp Val Gly Ser His Tyr Ile Trp Met
385 390 395 400

Ala Arg Tyr Leu Lys Ser Tyr Glu Pro Arg His Leu Leu Phe Ser Asn
405 410 415

[0099]

```
Gly Met Gln Thr Ile Gly Ile Ala Ile Thr Trp Ala Ile Ser Ala Ala
            420                 425                 430

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

Phe Leu Phe Ser Ala Gln Glu Leu Glu Thr Ala Val Arg Leu Lys Leu
    450                 455                 460

Pro Ile Val His Ile Ile Trp Asn Asp Gly His Tyr Asn Met Val Glu
465                 470                 475                 480

Phe Gln Glu Glu Met Lys Tyr Gly Arg Ser Ser Gly Val Asp Phe Gly
                485                 490                 495

Pro Val Asp Phe Val Lys Tyr Ala Glu Ser Phe Gly Ala Lys Gly Tyr
            500                 505                 510

Arg Ala Thr Ser Lys Ala Ala Phe Ala Ser Leu Leu Gln Glu Ala Leu
        515                 520                 525

Thr Gln Ala Val Asp Gly Pro Val Leu Ile Asp Val Pro Ile Asp Tyr
    530                 535                 540

Lys Asp Asn Ile Lys Leu Gly Glu Thr Ile Leu Pro Asp Glu Phe Tyr
545                 550                 555                 560
```

<210> 84
<211> 1665
<212> DNA
<213> 纤细弧菌 (Vibrio angustum)

<220>
<221> CDS
<222> (1)..(1665)

<400> 84

```
atg tcg gat aaa acc gtc tct ggt gct gaa ctg gtt gtt gaa act tta          48
Met Ser Asp Lys Thr Val Ser Gly Ala Glu Leu Val Val Glu Thr Leu
1               5                   10                  15

aat gca cat aac gtt cca cac att ttt ggt att cct gga gca aag gtg          96
Asn Ala His Asn Val Pro His Ile Phe Gly Ile Pro Gly Ala Lys Val
            20                  25                  30

gat gct gtt ttc gat gct gtt tgt gat aac gga cca gaa atc att att         144
Asp Ala Val Phe Asp Ala Val Cys Asp Asn Gly Pro Glu Ile Ile Ile
        35                  40                  45

tgt cat cat gaa caa aat gca gcg ttt atg gca gca gca act ggg cgt         192
Cys His His Glu Gln Asn Ala Ala Phe Met Ala Ala Ala Thr Gly Arg
    50                  55                  60

tta acg ggt aaa gca ggc att tgt tta gca acc tct gga cca ggc gca         240
Leu Thr Gly Lys Ala Gly Ile Cys Leu Ala Thr Ser Gly Pro Gly Ala
65                  70                  75                  80
```

[0100]

```
tca aac ctt gtc aca ggc gtt gca aca gcg aat agt gaa ggt gat cct      288
Ser Asn Leu Val Thr Gly Val Ala Thr Ala Asn Ser Glu Gly Asp Pro
                85                  90                  95

gtg gtt gca ctt gca ggt gct gta cct ctt tct atg tat tct cac aat      336
Val Val Ala Leu Ala Gly Ala Val Pro Leu Ser Met Tyr Ser His Asn
            100                 105                 110

act cat caa tcc atg gat acc cgt tca ctg ttt act cct atc acc aag      384
Thr His Gln Ser Met Asp Thr Arg Ser Leu Phe Thr Pro Ile Thr Lys
        115                 120                 125

ttt tca gca gaa gtg atg gat agc agc tcg gta tct gat gtt gta cat      432
Phe Ser Ala Glu Val Met Asp Ser Ser Ser Val Ser Asp Val Val His
    130                 135                 140

aaa gct ttt cgt att gca gag caa cct acc caa ggt gct agc ttt gtt      480
Lys Ala Phe Arg Ile Ala Glu Gln Pro Thr Gln Gly Ala Ser Phe Val
145                 150                 155                 160

agt cta ccg caa gat att cta act aac cgt att cct tac cag cca gta      528
Ser Leu Pro Gln Asp Ile Leu Thr Asn Arg Ile Pro Tyr Gln Pro Val
                165                 170                 175

caa cag cct aat cca att ttg ttc ggt ggt gca cac cca caa gct att      576
Gln Gln Pro Asn Pro Ile Leu Phe Gly Gly Ala His Pro Gln Ala Ile
            180                 185                 190

cgt cag gct gct gat cgc att aat gct gca aaa aat ccg gtg tta tta      624
Arg Gln Ala Ala Asp Arg Ile Asn Ala Ala Lys Asn Pro Val Leu Leu
        195                 200                 205

ctg ggc atg gat gca agc cag cct ttt gtt gct gat gct att cgc caa      672
Leu Gly Met Asp Ala Ser Gln Pro Phe Val Ala Asp Ala Ile Arg Gln
    210                 215                 220

cta ctc aaa caa aca cca att gcc gtt gtg aat acg ttt gcc gca gct      720
Leu Leu Lys Gln Thr Pro Ile Ala Val Val Asn Thr Phe Ala Ala Ala
225                 230                 235                 240

ggg gtt att tct cat gat tta tac aac tgc ttt tta ggt cgt gtt ggc      768
Gly Val Ile Ser His Asp Leu Tyr Asn Cys Phe Leu Gly Arg Val Gly
                245                 250                 255

tta ttt aaa aat caa ccc ggt gat att gca tta aac agt gca gat tta      816
Leu Phe Lys Asn Gln Pro Gly Asp Ile Ala Leu Asn Ser Ala Asp Leu
            260                 265                 270

atc att acc att ggc tac agc cca att gaa tac gat ccg att ctt tgg      864
Ile Ile Thr Ile Gly Tyr Ser Pro Ile Glu Tyr Asp Pro Ile Leu Trp
        275                 280                 285

aat aaa gat gca aac aca cca att att cat att ggt tat caa caa gca      912
Asn Lys Asp Ala Asn Thr Pro Ile Ile His Ile Gly Tyr Gln Gln Ala
    290                 295                 300

gat tta gaa att agc tat aac cct gtt tgt gaa gtt gtg ggt gac tta      960
Asp Leu Glu Ile Ser Tyr Asn Pro Val Cys Glu Val Val Gly Asp Leu
305                 310                 315                 320

gcg gtg tct gtc acg tct att gct tct gaa tta gat aag cga gaa tca     1008
Ala Val Ser Val Thr Ser Ile Ala Ser Glu Leu Asp Lys Arg Glu Ser
                325                 330                 335

tta gaa aat aac caa caa atc caa tta tta cgc cac gat tta caa cat     1056
```

[0101]

```
Leu Glu Asn Asn Gln Gln Ile Gln Leu Leu Arg His Asp Leu Gln His
            340                 345                 350

att atg cag atg ggg gta aat aaa acc tca aca aac ggc gtt cac ccg      1104
Ile Met Gln Met Gly Val Asn Lys Thr Ser Thr Asn Gly Val His Pro
        355                 360                 365

ctt cgt ttt gtt cat gag tta cgt cgc ttt gtt agt gac gac acc act      1152
Leu Arg Phe Val His Glu Leu Arg Arg Phe Val Ser Asp Asp Thr Thr
    370                 375                 380

gta tgt tgt gat gta ggc tct att tat att tgg atg gca cgt tac ttc      1200
Val Cys Cys Asp Val Gly Ser Ile Tyr Ile Trp Met Ala Arg Tyr Phe
385                 390                 395                 400

cac agc ttt gaa cct cgt cgt tta ttg ttc agc aat ggc caa caa aca      1248
His Ser Phe Glu Pro Arg Arg Leu Leu Phe Ser Asn Gly Gln Gln Thr
                405                 410                 415

ttg ggc gta gct tta cct tgg gca att gca gct tcc ctt ctt cac cct      1296
Leu Gly Val Ala Leu Pro Trp Ala Ile Ala Ala Ser Leu Leu His Pro
            420                 425                 430

aat gaa aaa gta att tcc atg tct ggt gat ggt ggc ttc cta ttc tca      1344
Asn Glu Lys Val Ile Ser Met Ser Gly Asp Gly Gly Phe Leu Phe Ser
        435                 440                 445

tca atg gaa tta gcc acg gcc gtt cgc cat aaa tgt aat atc gtt cac      1392
Ser Met Glu Leu Ala Thr Ala Val Arg His Lys Cys Asn Ile Val His
    450                 455                 460

ttt gtt tgg aca gat cac agt tat gac atg gtt aag atc caa cag ctt      1440
Phe Val Trp Thr Asp His Ser Tyr Asp Met Val Lys Ile Gln Gln Leu
465                 470                 475                 480

aaa aag tat ggt cga gag agt gcc gtc agc ttt ata ggt cct gat att      1488
Lys Lys Tyr Gly Arg Glu Ser Ala Val Ser Phe Ile Gly Pro Asp Ile
                485                 490                 495

gtt aag tac gca gaa agc ttc ggc gca cat ggt tta gcg atc aat act      1536
Val Lys Tyr Ala Glu Ser Phe Gly Ala His Gly Leu Ala Ile Asn Thr
            500                 505                 510

gcc gat gat att gag cct gtt atg cga aaa gct atg agc tta agt ggc      1584
Ala Asp Asp Ile Glu Pro Val Met Arg Lys Ala Met Ser Leu Ser Gly
        515                 520                 525

cca gta ttg gtc aac gtc aat gtt gat tat agc gat aac agt cgc cta      1632
Pro Val Leu Val Asn Val Asn Val Asp Tyr Ser Asp Asn Ser Arg Leu
    530                 535                 540

ctt gat caa ctt cat cca tgc caa caa gat taa                         1665
Leu Asp Gln Leu His Pro Cys Gln Gln Asp
545                 550
```

<210> 85
<211> 554
<212> PRT
<213> 纤细弧菌（Vibrio angustum）

<400> 85

```
Met Ser Asp Lys Thr Val Ser Gly Ala Glu Leu Val Val Glu Thr Leu
1               5                   10                  15
```

[0102]

Asn Ala His Asn Val Pro His Ile Phe Gly Ile Pro Gly Ala Lys Val
20 25 30

Asp Ala Val Phe Asp Ala Val Cys Asp Asn Gly Pro Glu Ile Ile Ile
35 40 45

Cys His His Glu Gln Asn Ala Ala Phe Met Ala Ala Ala Thr Gly Arg
50 55 60

Leu Thr Gly Lys Ala Gly Ile Cys Leu Ala Thr Ser Gly Pro Gly Ala
65 70 75 80

Ser Asn Leu Val Thr Gly Val Ala Thr Ala Asn Ser Glu Gly Asp Pro
85 90 95

Val Val Ala Leu Ala Gly Ala Val Pro Leu Ser Met Tyr Ser His Asn
100 105 110

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

Phe Ser Ala Glu Val Met Asp Ser Ser Ser Val Ser Asp Val Val His
130 135 140

Lys Ala Phe Arg Ile Ala Glu Gln Pro Thr Gln Gly Ala Ser Phe Val
145 150 155 160

Ser Leu Pro Gln Asp Ile Leu Thr Asn Arg Ile Pro Tyr Gln Pro Val
165 170 175

Gln Gln Pro Asn Pro Ile Leu Phe Gly Gly Ala His Pro Gln Ala Ile
180 185 190

Arg Gln Ala Ala Asp Arg Ile Asn Ala Ala Lys Asn Pro Val Leu Leu
195 200 205

Leu Gly Met Asp Ala Ser Gln Pro Phe Val Ala Asp Ala Ile Arg Gln
210 215 220

Leu Leu Lys Gln Thr Pro Ile Ala Val Val Asn Thr Phe Ala Ala Ala
225 230 235 240

Gly Val Ile Ser His Asp Leu Tyr Asn Cys Phe Leu Gly Arg Val Gly
245 250 255

Leu Phe Lys Asn Gln Pro Gly Asp Ile Ala Leu Asn Ser Ala Asp Leu
260 265 270

Ile Ile Thr Ile Gly Tyr Ser Pro Ile Glu Tyr Asp Pro Ile Leu Trp

[0103]

275 280 285

Asn Lys Asp Ala Asn Thr Pro Ile Ile His Ile Gly Tyr Gln Gln Ala
290 295 300

Asp Leu Glu Ile Ser Tyr Asn Pro Val Cys Glu Val Val Gly Asp Leu
305 310 315 320

Ala Val Ser Val Thr Ser Ile Ala Ser Glu Leu Asp Lys Arg Glu Ser
325 330 335

Leu Glu Asn Asn Gln Gln Ile Gln Leu Leu Arg His Asp Leu Gln His
340 345 350

Ile Met Gln Met Gly Val Asn Lys Thr Ser Thr Asn Gly Val His Pro
355 360 365

Leu Arg Phe Val His Glu Leu Arg Arg Phe Val Ser Asp Asp Thr Thr
370 375 380

Val Cys Cys Asp Val Gly Ser Ile Tyr Ile Trp Met Ala Arg Tyr Phe
385 390 395 400

His Ser Phe Glu Pro Arg Arg Leu Leu Phe Ser Asn Gly Gln Gln Thr
405 410 415

Leu Gly Val Ala Leu Pro Trp Ala Ile Ala Ala Ser Leu Leu His Pro
420 425 430

Asn Glu Lys Val Ile Ser Met Ser Gly Asp Gly Gly Phe Leu Phe Ser
435 440 445

Ser Met Glu Leu Ala Thr Ala Val Arg His Lys Cys Asn Ile Val His
450 455 460

Phe Val Trp Thr Asp His Ser Tyr Asp Met Val Lys Ile Gln Gln Leu
465 470 475 480

Lys Lys Tyr Gly Arg Glu Ser Ala Val Ser Phe Ile Gly Pro Asp Ile
485 490 495

Val Lys Tyr Ala Glu Ser Phe Gly Ala His Gly Leu Ala Ile Asn Thr
500 505 510

Ala Asp Asp Ile Glu Pro Val Met Arg Lys Ala Met Ser Leu Ser Gly
515 520 525

Pro Val Leu Val Asn Val Asn Val Asp Tyr Ser Asp Asn Ser Arg Leu
530 535 540

[0104]

Leu Asp Gln Leu His Pro Cys Gln Gln Asp
545 550

<210> 86
<211> 1689
<212> DNA
<213> 蜡状芽孢杆菌 (Bacillus cereus)

<400> 86
ttgagtacag gtgtaaaagc aaacgacgtg aagacaaaaa caaaaggagc agatcttgtt 60

gttgattgtt taattaaaca aggtgttaca catgttttcg gtattccagg agcaaagatt 120

gactctgtat ttgatgtact gcaagaaaga ggaccagagt taattgtttg tcgtcatgaa 180

caaaatgcag catttatggc agctgctatt ggtagattaa caggaaaacc gggcgtatgt 240

cttgtaactt caggaccagg gacatcaaat ttagcgacag gtcttgttac tgcgaatgcg 300

gagagtgatc ccgttgttgc tttagctggt gcagttccgc gtacggatcg attaaaacgt 360

acacatcaat ctatggataa tgctgcacta ttcgaaccaa tcacaaaata tagcgtagaa 420

gtagagcatc ctgataatgt gccagaagca ttatcaaatg cattccgaag tgcgacttct 480

acaaatccag gagcaacttt agtaagtttg ccgcaagacg ttatgactgc ggaaacgact 540

gtagagtcta tcggtgcgct ttctaagcca cagcttggaa tcgctcccac acatgatatt 600

acatatgtag tagataaaat aaaagcagcg aaattaccag ttattttact cggtatgaga 660

gcgagcacaa atgaagtgac gaaagccgtt cgtaaattaa ttgcggatac agaacttcct 720

gtcgttgaaa catatcaagc ggctggtgcc atttcacgtg agttagaaga tcatttcttc 780

ggccgtgttg gactattccg taaccaacca ggtgatattt tactagaaga ggcagatctt 840

gttatttcta tcggttatga tccaattgag tatgatccaa agttctggaa taaacttgga 900

gacagaacga ttattcatct tgatgaccat caagcagata tagatcatga ttaccaacca 960

gagcgtgaat taattggtga tattgcctta acagtaaata gcatcgcaga aaagttaccg 1020

aaacttgtgt taagtacgaa atcagaagca gtgttagaac gattacgcgc gaaattatca 1080

gaacaagcag aagttccaaa tcgtccttca gaaggtgtta cacatccgct tcaagtgatt 1140

cgtacacttc gttctttaat tagtgacgac acaaccgtta catgtgacat cggttcccat 1200

tctatttgga tggcgagatg tttccgttct tatgaaccac gtagattatt atttagtaac 1260

ggtatgcaga cgttaggtgt tgcacttcct tgggcaattg ctgctacttt agtagaacca 1320

ggtaaaaaag tagtttccgt atcaggtgac ggtggtttct tattctcagc gatggagtta 1380

gaaacggcgg tacgtttaaa ttctccaatc gtccatcttg tttggagaga cggcacatat 1440

gatatggttg cattccaaca aatgatgaaa tacggcagaa catcagctac agagtttggt 1500

gatgttgatc ttgttaaata tgcggaaagt ttcggggcgt taggtcttcg tgttaacacg 1560

cctgatgaat tagaaggggt attgaaagaa gcactagcag cagacggccc tgtcattatt 1620

gatattccaa ttgactatcg tgacaacatt aaattaagcg aaaaattatt accaaaccaa 1680

[0105]

ttaaactaa 1689

<210> 87
<211> 562
<212> PRT
<213> 蜡状芽孢杆菌（Bacillus cereus）

<400> 87

Met Ser Thr Gly Val Lys Ala Asn Asp Val Lys Thr Lys Thr Lys Gly
1 5 10 15

Ala Asp Leu Val Val Asp Cys Leu Ile Lys Gln Gly Val Thr His Val
20 25 30

Phe Gly Ile Pro Gly Ala Lys Ile Asp Ser Val Phe Asp Val Leu Gln
35 40 45

Glu Arg Gly Pro Glu Leu Ile Val Cys Arg His Glu Gln Asn Ala Ala
50 55 60

Phe Met Ala Ala Ala Ile Gly Arg Leu Thr Gly Lys Pro Gly Val Cys
65 70 75 80

Leu Val Thr Ser Gly Pro Gly Thr Ser Asn Leu Ala Thr Gly Leu Val
85 90 95

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

Pro Arg Thr Asp Arg Leu Lys Arg Thr His Gln Ser Met Asp Asn Ala
115 120 125

Ala Leu Phe Glu Pro Ile Thr Lys Tyr Ser Val Glu Val Glu His Pro
130 135 140

Asp Asn Val Pro Glu Ala Leu Ser Asn Ala Phe Arg Ser Ala Thr Ser
145 150 155 160

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

Ala Glu Thr Thr Val Glu Ser Ile Gly Ala Leu Ser Lys Pro Gln Leu
180 185 190

Gly Ile Ala Pro Thr His Asp Ile Thr Tyr Val Val Asp Lys Ile Lys
195 200 205

Ala Ala Lys Leu Pro Val Ile Leu Leu Gly Met Arg Ala Ser Thr Asn
210 215 220

[0106]

Glu Val Thr Lys Ala Val Arg Lys Leu Ile Ala Asp Thr Glu Leu Pro
225 230 235 240

Val Val Glu Thr Tyr Gln Ala Ala Gly Ala Ile Ser Arg Glu Leu Glu
245 250 255

Asp His Phe Phe Gly Arg Val Gly Leu Phe Arg Asn Gln Pro Gly Asp
260 265 270

Ile Leu Leu Glu Glu Ala Asp Leu Val Ile Ser Ile Gly Tyr Asp Pro
275 280 285

Ile Glu Tyr Asp Pro Lys Phe Trp Asn Lys Leu Gly Asp Arg Thr Ile
290 295 300

Ile His Leu Asp Asp His Gln Ala Asp Ile Asp His Asp Tyr Gln Pro
305 310 315 320

Glu Arg Glu Leu Ile Gly Asp Ile Ala Leu Thr Val Asn Ser Ile Ala
325 330 335

Glu Lys Leu Pro Lys Leu Val Leu Ser Thr Lys Ser Glu Ala Val Leu
340 345 350

Glu Arg Leu Arg Ala Lys Leu Ser Glu Gln Ala Glu Val Pro Asn Arg
355 360 365

Pro Ser Glu Gly Val Thr His Pro Leu Gln Val Ile Arg Thr Leu Arg
370 375 380

Ser Leu Ile Ser Asp Asp Thr Thr Val Thr Cys Asp Ile Gly Ser His
385 390 395 400

Ser Ile Trp Met Ala Arg Cys Phe Arg Ser Tyr Glu Pro Arg Arg Leu
405 410 415

Leu Phe Ser Asn Gly Met Gln Thr Leu Gly Val Ala Leu Pro Trp Ala
420 425 430

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

Gly Asp Gly Gly Phe Leu Phe Ser Ala Met Glu Leu Glu Thr Ala Val
450 455 460

Arg Leu Asn Ser Pro Ile Val His Leu Val Trp Arg Asp Gly Thr Tyr
465 470 475 480

Asp Met Val Ala Phe Gln Gln Met Met Lys Tyr Gly Arg Thr Ser Ala

[0107]

485 490 495

Thr Glu Phe Gly Asp Val Asp Leu Val Lys Tyr Ala Glu Ser Phe Gly
500 505 510

Ala Leu Gly Leu Arg Val Asn Thr Pro Asp Glu Leu Glu Gly Val Leu
515 520 525

Lys Glu Ala Leu Ala Ala Asp Gly Pro Val Ile Ile Asp Ile Pro Ile
530 535 540

Asp Tyr Arg Asp Asn Ile Lys Leu Ser Glu Lys Leu Leu Pro Asn Gln
545 550 555 560

Leu Asn

<210> 88
<211> 1014
<212> DNA
<213> 荧光假单胞菌（Pseudomonas fluorescens）

<400> 88
atgaaagttt tctacgataa agactgcgac ctgtcgatca tccaaggtaa gaaagttgcc 60

atcatcggct acggttccca gggccacgct caagcatgca acctgaagga ttccggcgta 120

gacgtgactg ttggcctgcg taaaggctcg gctaccgttg ccaaggctga agcccacggc 180

ttgaaagtga ccgacgttgc tgcagccgtt gccggtgccg acttggtcat gatcctgacc 240

ccggacgagt tccagtccca gctgtacaag aacgaaatcg agccgaacat caagaagggc 300

gccactctgg ccttctccca cggcttcgcg atccactaca accaggttgt gcctcgtgcc 360

gacctcgacg tgatcatgat cgcgccgaag gctccaggcc acaccgtacg ttccgagttc 420

gtcaagggcg gtggtattcc tgacctgatc gcgatctacc aggacgcttc cggcaacgcc 480

aagaacgttg ccctgtccta cgccgcaggc gtgggcggcg gccgtaccgg catcatcgaa 540

accaccttca aggacgagac tgaaaccgac ctgttcggtg agcaggctgt tctgtgtggc 600

ggtaccgtcg agctggtcaa agccggtttc gaaaccctgg ttgaagctgg ctacgctcca 660

gaaatggcct acttcgagtg cctgcacgaa ctgaagctga tcgttgacct catgtacgaa 720

ggcggtatcg ccaacatgaa ctactcgatc tccaacaacg ctgaatacgg cgagtacgtg 780

actggtccag aagtcatcaa cgccgaatcc cgtcaggcca tgcgcaatgc tctgaagcgc 840

atccaggacg gcgaatacgc gaagatgttc atcagcgaag gcgctaccgg ctacccatcg 900

atgaccgcca agcgtcgtaa caacgctgct cacggtatcg aaatcatcgg cgagcaactg 960

cgctcgatga tgccttggat cggtgccaac aaaatcgtcg acaaagccaa gaac 1014

<210> 89
<211> 338

[0108]

<212> PRT
<213> 荧光假单胞菌（Pseudomonas fluorescens）

<400> 89

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly
1 5 10 15

Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala Gln Ala
20 25 30

Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Arg Lys
35 40 45

Gly Ser Ala Thr Val Ala Lys Ala Glu Ala His Gly Leu Lys Val Thr
50 55 60

Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Thr
65 70 75 80

Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn
85 90 95

Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His
100 105 110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala
115 120 125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly
130 135 140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Asn Ala
145 150 155 160

Lys Asn Val Ala Leu Ser Tyr Ala Ala Gly Val Gly Gly Gly Arg Thr
165 170 175

Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr
210 215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu
225 230 235 240

Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr

[0109]

245 250 255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln
260 265 270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys
275 280 285

Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys
290 295 300

Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu
305 310 315 320

Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala
325 330 335

Lys Asn

<210> 90
<211> 1014
<212> DNA
<213> 人工序列

<220>
<223> 经优化以在植物乳杆菌（Lactobacillus plantarum）中表达的PF5-ilvC编码序列

<400> 90
atgaaagtct tttatgataa ggattgtgat ttgtcaatta ttcaaggtaa gaaggtcgct 60

attattggtt atggttcaca aggtcacgcc caagcctgta atttgaagga tagtggtgtt 120

gatgttacgg ttggtttacg gaagggtagt gctacggttg cgaaagccga agcgcatggt 180

ttgaaggtta cggatgttgc agctgccgtc gccggtgcgg atttggttat gattttaacg 240

ccagatgaat ttcaaagtca attatataaa aatgaaattg aaccaaatat taaaaaaggt 300

gctacgttag cttttagtca tggttttgcg attcattata atcaagttgt cccacgggct 360

gatttagatg tcattatgat tgcaccaaaa gctccaggtc atacggttcg tagtgaattt 420

gtcaagggtg gtggtattcc agatttgatt gcaatttatc aagatgcaag tggtaatgcc 480

aaaaatgtcg ctttaagtta tgcggccggt gttggtggtg gtcggacggg tattattgaa 540

acgacgttta aagatgaaac ggaaacggat ttgtttggtg aacaagccgt cttatgtggt 600

ggtacggttg aattagttaa ggcgggtttt gaaacgttgg ttgaagctgg ttatgcacca 660

gaaatggcgt attttgaatg tttacatgaa ttaaaattaa ttgttgattt gatgtatgaa 720

ggtggtattg caaatatgaa ttatagtatt agtaataatg cagaatatgg tgaatatgtt 780

acgggtccag aagttattaa tgcagaaagt cggcaagcaa tgcgtaatgc cttaaaacgg 840

attcaagatg gtgaatatgc caaaatgttt atttcagaag gtgcgacggg ttatccaagt 900

[0110]

atgacggcta agcggcggaa taatgccgcc catggtattg aaattattgg tgaacaatta 960

cggagtatga tgccatggat tggtgctaat aaaattgttg ataaggcaaa gaat 1014

<210> 91
<211> 1014
<212> DNA
<213> 人工序列

<220>
<223> 经密码子优化以在啤酒糖酵母（Saccharomyces cerevisiae）中表达的PF5 ilvC

<400> 91
atgaaggtgt tttacgataa agactgcgat ctgagcatca tccagggaaa gaaggttgct 60

attataggat atggttccca aggacacgca caagcctgta acttgaaaga ttctggggtc 120

gacgtgacag taggtctgag aaaaggtagt gctaccgttg caaaggctga agcacatggc 180

ttgaaagtca cagatgttgc agcggctgtt gccggcgctg atttagtcat gattttaacg 240

ccagatgaat ttcaatcgca attgtacaaa aatgaaatag aaccaaacat taagaagggc 300

gctaccttgg ccttcagtca tggatttgcc attcattaca atcaagtagt ccccagggca 360

gatttggacg ttattatgat tgcacctaag gctccggggc atactgttag gagcgaattt 420

gttaagggtg gtggtattcc agatttgatc gctatatacc aagacgcaag cggaaacgct 480

aagaatgtag ctttaagcta cgcagcagga gttggtggcg ggagaacggg tataatagaa 540

accactttta aagacgagac tgagacagat ttatttggag aacaagcggt tctgtgcgga 600

ggaactgttg aattggttaa agcaggcttt gagacgcttg tcgaagcagg gtacgctccc 660

gaaatggcat acttcgaatg tctacatgaa ttgaagttga tagtagactt aatgtatgaa 720

ggtggtatag ctaatatgaa ctattccatt tcaaataatg cagaatatgg tgagtatgtc 780

accggacctg aagtcattaa cgcagaatca agacaagcca tgagaaatgc cttgaaacgt 840

atccaggacg gtgaatacgc taagatgttc ataagtgaag gcgctacggg ttacccgagt 900

atgactgcta aaagaagaaa caatgcagca catggtatcg aaattattgg tgaacagtta 960

aggtctatga tgccctggat cggtgctaat aagatcgtag acaaggcgaa aaat 1014

<210> 92
<211> 1047
<212> DNA
<213> 啤酒糖酵母（Saccharomyces cerevisiae）

<400> 92
atgttgaagc aaatcaactt cggtggtact gttgaaaccg tctacgaaag agctgactgg 60

ccaagagaaa agttgttgga ctacttcaag aacgacactt ttgctttgat cggttacggt 120

tcccaaggtt acggtcaagg tttgaacttg agagacaacg gtttgaacgt tatcattggt 180

gtccgtaaag atggtgcttc ttggaaggct gccatcgaag acggttgggt tccaggcaag 240

aacttgttca ctgttgaaga tgctatcaag agaggtagtt acgttatgaa cttgttgtcc 300

[0111]

gatgccgctc aatcagaaac ctggcctgct atcaagccat tgttgaccaa gggtaagact 360

ttgtacttct cccacggttt ctccccagtc ttcaaggact tgactcacgt tgaaccacca 420

aaggacttag atgttatctt ggttgctcca aagggttccg gtagaactgt cagatctttg 480

ttcaaggaag gtcgtggtat taactcttct tacgccgtct ggaacgatgt caccggtaag 540

gctcacgaaa aggcccaagc tttggccgtt gccattggtt ccggttacgt ttaccaaacc 600

actttcgaaa gagaagtcaa ctctgacttg tacggtgaaa gaggttgttt aatgggtggt 660

atccacggta tgttcttggc tcaatacgac gtcttgagag aaaacggtca ctccccatct 720

gaagctttca acgaaaccgt cgaagaagct acccaatctc tatacccatt gatcggtaag 780

tacggtatgg attacatgta cgatgcttgt tccaccaccg ccagaagagg tgctttggac 840

tggtacccaa tcttcaagaa tgctttgaag cctgttttcc aagacttgta cgaatctacc 900

aagaacggta ccgaaaccaa gagatctttg gaattcaact ctcaacctga ctacagagaa 960

aagctagaaa aggaattaga caccatcaga aacatggaaa tctggaaggt tggtaaggaa 1020

gtcagaaagt tgagaccaga aaaccaa 1047

<210> 93
<211> 349
<212> PRT
<213> 啤酒糖酵母 (Saccharomyces cerevisiae)

<400> 93

Met Leu Lys Gln Ile Asn Phe Gly Gly Thr Val Glu Thr Val Tyr Glu
1 5 10 15

Arg Ala Asp Trp Pro Arg Glu Lys Leu Leu Asp Tyr Phe Lys Asn Asp
20 25 30

Thr Phe Ala Leu Ile Gly Tyr Gly Ser Gln Gly Tyr Gly Gln Gly Leu
35 40 45

Asn Leu Arg Asp Asn Gly Leu Asn Val Ile Ile Gly Val Arg Lys Asp
50 55 60

Gly Ala Ser Trp Lys Ala Ala Ile Glu Asp Gly Trp Val Pro Gly Lys
65 70 75 80

Asn Leu Phe Thr Val Glu Asp Ala Ile Lys Arg Gly Ser Tyr Val Met
85 90 95

Asn Leu Leu Ser Asp Ala Ala Gln Ser Glu Thr Trp Pro Ala Ile Lys
100 105 110

Pro Leu Leu Thr Lys Gly Lys Thr Leu Tyr Phe Ser His Gly Phe Ser
115 120 125

[0112]

Pro Val Phe Lys Asp Leu Thr His Val Glu Pro Pro Lys Asp Leu Asp
130 135 140

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

Phe Lys Glu Gly Arg Gly Ile Asn Ser Ser Tyr Ala Val Trp Asn Asp
165 170 175

Val Thr Gly Lys Ala His Glu Lys Ala Gln Ala Leu Ala Val Ala Ile
180 185 190

Gly Ser Gly Tyr Val Tyr Gln Thr Thr Phe Glu Arg Glu Val Asn Ser
195 200 205

Asp Leu Tyr Gly Glu Arg Gly Cys Leu Met Gly Gly Ile His Gly Met
210 215 220

Phe Leu Ala Gln Tyr Asp Val Leu Arg Glu Asn Gly His Ser Pro Ser
225 230 235 240

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

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

Thr Ala Arg Arg Gly Ala Leu Asp Trp Tyr Pro Ile Phe Lys Asn Ala
275 280 285

Leu Lys Pro Val Phe Gln Asp Leu Tyr Glu Ser Thr Lys Asn Gly Thr
290 295 300

Glu Thr Lys Arg Ser Leu Glu Phe Asn Ser Gln Pro Asp Tyr Arg Glu
305 310 315 320

Lys Leu Glu Lys Glu Leu Asp Thr Ile Arg Asn Met Glu Ile Trp Lys
325 330 335

Val Gly Lys Glu Val Arg Lys Leu Arg Pro Glu Asn Gln
340 345

<210> 94
<211> 1713
<212> DNA
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 94
atggaattca aatataacgg aaaagttgaa tcaatagagc tcaataagta ttcaaaaaca 60

ttgacacaag acccaacaca gccagcgacc caagccatgc actatggcat tggttttaaa 120

[0113]

gatgaggact tcaaaaaagc tcaggtcgga atcgtcagca tggattggga cggaaatccc 180

tgtaacatgc acttgggcac actcggtagt aaaatcaaaa attctgtcaa tcaaactgac 240

ggactgattg ggcttcaatt tcacacgatt ggggtttctg acggaattgc caacggaaag 300

cttggcatga gatattcctt ggtcagtcgt gaagttattg ctgacagtat tgaaaccaac 360

gctggcgccg aatattacga tgcaattgtc gctgttcccg gttgtgacaa aaatatgcca 420

ggctcaatca ttggcatggc tcggctcaat cgtccgtcaa ttatggttta tggtggaacg 480

attgaacatg gcgaatacaa aggcgaaaag ttaaatattg tttcggcttt tgaagcgctt 540

ggacaaaaaa tcactggaaa tatttccgag gaagattatc acggcgtcat ttgtaatgcc 600

attccgggac agggtgcttg tgggggcatg tatacagcaa atacactggc ttcggcaatt 660

gaaactttgg gaatgagttt gccttattcg gcttcaaatc cagcggtcag tcaagaaaaa 720

gaagacgaat gtgatgaaat tggtctggca atcaaaaatt tgctagaaaa agacatcaaa 780

ccaagcgata tcatgaccaa ggaagctttt gaaaatgcca taacgatcgt catggttctc 840

ggtggttcaa ctaatgctgt gcttcatatc attgccatgg ctaatgccat cggtgtcgaa 900

attacgcaag atgattttca acgtatttcc gatgtcacgc ctgtgcttgg cgacttcaag 960

ccaagtggca agtacatgat ggaagatttg cacaaaattg gtggcgtgcc tgctgttttg 1020

aaatatttgc tcaaagaggg caagcttcat ggcgactgtt tgacagttac tggtaaaact 1080

ctagctgaaa atgttgaaac agcactggat ttggactttg acagccaaga cattattcga 1140

ccacttgaaa atcctatcaa agcaacaggt catttgcaaa ttctctatgg caatcttgct 1200

gaaggtggtt ctgtggcaaa aatttctgga aaagaagggg aatttttcaa aggaacagct 1260

cgtgtctttg atggcgaaca acattttatt gacggaattg agtcaggtcg tttgcacgca 1320

ggagatgtcg ctgtcattcg taatatcggt ccagtcggag gcccaggaat gccagaaatg 1380

ttaaaaccga cctcagcctt gattggagca ggtcttggaa aatcttgtgc tttgattact 1440

gacggacgtt tctctggtgg cacacatggc tttgtggttg gccacattgt ccccgaagcg 1500

gttgagggtg gattgattgg cttggtcgaa gatgatgata ttatcgagat tgatgcggtc 1560

aataacagca ttagtttgaa agttgctgac gatgagattg ctagacgacg tgccaattat 1620

caaaaacctg ctcctaaagc aacgcgtggg gttcttgcta aatttgctaa actcacgcgc 1680

ccagccagtg aaggctgtgt gactgattta tag 1713

<210> 95
<211> 570
<212> PRT
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 95

Met Glu Phe Lys Tyr Asn Gly Lys Val Glu Ser Ile Glu Leu Asn Lys
1 5 10 15

[0114]

Tyr Ser Lys Thr Leu Thr Gln Asp Pro Thr Gln Pro Ala Thr Gln Ala
20 25 30

Met His Tyr Gly Ile Gly Phe Lys Asp Glu Asp Phe Lys Lys Ala Gln
35 40 45

Val Gly Ile Val Ser Met Asp Trp Asp Gly Asn Pro Cys Asn Met His
50 55 60

Leu Gly Thr Leu Gly Ser Lys Ile Lys Asn Ser Val Asn Gln Thr Asp
65 70 75 80

Gly Leu Ile Gly Leu Gln Phe His Thr Ile Gly Val Ser Asp Gly Ile
85 90 95

Ala Asn Gly Lys Leu Gly Met Arg Tyr Ser Leu Val Ser Arg Glu Val
100 105 110

Ile Ala Asp Ser Ile Glu Thr Asn Ala Gly Ala Glu Tyr Tyr Asp Ala
115 120 125

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

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

Ile Glu His Gly Glu Tyr Lys Gly Glu Lys Leu Asn Ile Val Ser Ala
165 170 175

Phe Glu Ala Leu Gly Gln Lys Ile Thr Gly Asn Ile Ser Glu Glu Asp
180 185 190

Tyr His Gly Val Ile Cys Asn Ala Ile Pro Gly Gln Gly Ala Cys Gly
195 200 205

Gly Met Tyr Thr Ala Asn Thr Leu Ala Ser Ala Ile Glu Thr Leu Gly
210 215 220

Met Ser Leu Pro Tyr Ser Ala Ser Asn Pro Ala Val Ser Gln Glu Lys
225 230 235 240

Glu Asp Glu Cys Asp Glu Ile Gly Leu Ala Ile Lys Asn Leu Leu Glu
245 250 255

Lys Asp Ile Lys Pro Ser Asp Ile Met Thr Lys Glu Ala Phe Glu Asn
260 265 270

Ala Ile Thr Ile Val Met Val Leu Gly Gly Ser Thr Asn Ala Val Leu

[0115]

275 280 285

His Ile Ile Ala Met Ala Asn Ala Ile Gly Val Glu Ile Thr Gln Asp
290 295 300

Asp Phe Gln Arg Ile Ser Asp Val Thr Pro Val Leu Gly Asp Phe Lys
305 310 315 320

Pro Ser Gly Lys Tyr Met Met Glu Asp Leu His Lys Ile Gly Gly Val
325 330 335

Pro Ala Val Leu Lys Tyr Leu Leu Lys Glu Gly Lys Leu His Gly Asp
340 345 350

Cys Leu Thr Val Thr Gly Lys Thr Leu Ala Glu Asn Val Glu Thr Ala
355 360 365

Leu Asp Leu Asp Phe Asp Ser Gln Asp Ile Ile Arg Pro Leu Glu Asn
370 375 380

Pro Ile Lys Ala Thr Gly His Leu Gln Ile Leu Tyr Gly Asn Leu Ala
385 390 395 400

Glu Gly Gly Ser Val Ala Lys Ile Ser Gly Lys Glu Gly Glu Phe Phe
405 410 415

Lys Gly Thr Ala Arg Val Phe Asp Gly Glu Gln His Phe Ile Asp Gly
420 425 430

Ile Glu Ser Gly Arg Leu His Ala Gly Asp Val Ala Val Ile Arg Asn
435 440 445

Ile Gly Pro Val Gly Gly Pro Gly Met Pro Glu Met Leu Lys Pro Thr
450 455 460

Ser Ala Leu Ile Gly Ala Gly Leu Gly Lys Ser Cys Ala Leu Ile Thr
465 470 475 480

Asp Gly Arg Phe Ser Gly Gly Thr His Gly Phe Val Val Gly His Ile
485 490 495

Val Pro Glu Ala Val Glu Gly Gly Leu Ile Gly Leu Val Glu Asp Asp
500 505 510

Asp Ile Ile Glu Ile Asp Ala Val Asn Asn Ser Ile Ser Leu Lys Val
515 520 525

Ala Asp Asp Glu Ile Ala Arg Arg Arg Ala Asn Tyr Gln Lys Pro Ala
530 535 540

[0116]

Pro Lys Ala Thr Arg Gly Val Leu Ala Lys Phe Ala Lys Leu Thr Arg
545 550 555 560

Pro Ala Ser Glu Gly Cys Val Thr Asp Leu
565 570

<210> 96
<211> 1713
<212> DNA
<213> 变异链球菌 (Streptococcus mutans)

<400> 96
atgactgaca aaaaaactct taaagactta agaaatcgta gttctgttta cgattcaatg 60

gttaaatcac ctaatcgtgc tatgttgcgt gcaactggta tgcaagatga agactttgaa 120

aaacctatcg tcggtgtcat ttcaacttgg gctgaaaaca caccttgtaa tatccactta 180

catgactttg gtaaactagc caaagtcggt gttaaggaag ctggtgcttg gccagttcag 240

ttcggaacaa tcacggtttc tgatggaatc gccatgggaa cccaaggaat gcgtttctcc 300

ttgacatctc gtgatattat tgcagattct attgaagcag ccatgggagg tcataatgcg 360

gatgcttttg tagccattgg cggttgtgat aaaaacatgc ccggttctgt tatcgctatg 420

gctaacatgg atatcccagc catttttgct tacggcggaa caattgcacc tggtaattta 480

gacggcaaag atatcgattt agtctctgtc tttgaaggtg tcggccattg gaaccacggc 540

gatatgacca aagaagaagt taaagctttg gaatgtaatg cttgtcccgg tcctggaggc 600

tgcggtggta tgtatactgc taacacaatg gcgacagcta ttgaagtttt gggacttagc 660

cttccgggtt catcttctca cccggctgaa tccgcagaaa agaaagcaga tattgaagaa 720

gctggtcgcg ctgttgtcaa aatgctcgaa atgggcttaa aaccttctga cattttaacg 780

cgtgaagctt ttgaagatgc tattactgta actatggctc tgggaggttc aaccaactca 840

acccttcacc tcttagctat tgcccatgct gctaatgtgg aattgacact tgatgatttc 900

aatactttcc aagaaaaagt tcctcatttg gctgatttga aaccttctgg tcaatatgta 960

ttccaagacc tttacaaggt cggaggggta ccagcagtta tgaaatatct ccttaaaaat 1020

ggcttccttc atggtgaccg tatcacttgt actggcaaaa cagtcgctga aaatttgaag 1080

gcttttgatg atttaacacc tggtcaaaag gttattatgc cgcttgaaaa tcctaaacgt 1140

gaagatggtc cgctcattat tctccatggt aacttggctc cagacggtgc cgttgccaaa 1200

gtttctggtg taaaagtgcg tcgtcatgtc ggtcctgcta aggtctttaa ttctgaagaa 1260

gaagccattg aagctgtctt gaatgatgat attgttgatg gtgatgttgt tgtcgtacgt 1320

tttgtaggac caaagggcgg tcctggtatg cctgaaatgc tttccctttc atcaatgatt 1380

gttggtaaag ggcaaggtga aaaagttgcc cttctgacag atggccgctt ctcaggtggt 1440

acttatggtc ttgtcgtggg tcatatcgct cctgaagcac aagatggcgg tccaatcgcc 1500

tacctgcaaa caggagacat agtcactatt gaccaagaca ctaaggaatt acactttgat 1560

[0117]

atctccgatg aagagttaaa acatcgtcaa gagaccattg aattgccacc gctctattca 1620

cgcggtatcc ttggtaaata tgctcacatc gtttcgtctg cttctagggg agccgtaaca 1680

gacttttgga agcctgaaga aactggcaaa aaa 1713

<210> 97
<211> 571
<212> PRT
<213> 变异链球菌（Streptococcus mutans）

<400> 97

Met Thr Asp Lys Lys Thr Leu Lys Asp Leu Arg Asn Arg Ser Ser Val
1 5 10 15

Tyr Asp Ser Met Val Lys Ser Pro Asn Arg Ala Met Leu Arg Ala Thr
20 25 30

Gly Met Gln Asp Glu Asp Phe Glu Lys Pro Ile Val Gly Val Ile Ser
35 40 45

Thr Trp Ala Glu Asn Thr Pro Cys Asn Ile His Leu His Asp Phe Gly
50 55 60

Lys Leu Ala Lys Val Gly Val Lys Glu Ala Gly Ala Trp Pro Val Gln
65 70 75 80

Phe Gly Thr Ile Thr Val Ser Asp Gly Ile Ala Met Gly Thr Gln Gly
85 90 95

Met Arg Phe Ser Leu Thr Ser Arg Asp Ile Ile Ala Asp Ser Ile Glu
100 105 110

Ala Ala Met Gly Gly His Asn Ala Asp Ala Phe Val Ala Ile Gly Gly
115 120 125

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

Ile Pro Ala Ile Phe Ala Tyr Gly Gly Thr Ile Ala Pro Gly Asn Leu
145 150 155 160

Asp Gly Lys Asp Ile Asp Leu Val Ser Val Phe Glu Gly Val Gly His
165 170 175

Trp Asn His Gly Asp Met Thr Lys Glu Glu Val Lys Ala Leu Glu Cys
180 185 190

Asn Ala Cys Pro Gly Pro Gly Gly Cys Gly Gly Met Tyr Thr Ala Asn
195 200 205

[0118]

Thr Met Ala Thr Ala Ile Glu Val Leu Gly Leu Ser Leu Pro Gly Ser
210 215 220

Ser Ser His Pro Ala Glu Ser Ala Glu Lys Lys Ala Asp Ile Glu Glu
225 230 235 240

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

Asp Ile Leu Thr Arg Glu Ala Phe Glu Asp Ala Ile Thr Val Thr Met
260 265 270

Ala Leu Gly Gly Ser Thr Asn Ser Thr Leu His Leu Leu Ala Ile Ala
275 280 285

His Ala Ala Asn Val Glu Leu Thr Leu Asp Asp Phe Asn Thr Phe Gln
290 295 300

Glu Lys Val Pro His Leu Ala Asp Leu Lys Pro Ser Gly Gln Tyr Val
305 310 315 320

Phe Gln Asp Leu Tyr Lys Val Gly Gly Val Pro Ala Val Met Lys Tyr
325 330 335

Leu Leu Lys Asn Gly Phe Leu His Gly Asp Arg Ile Thr Cys Thr Gly
340 345 350

Lys Thr Val Ala Glu Asn Leu Lys Ala Phe Asp Asp Leu Thr Pro Gly
355 360 365

Gln Lys Val Ile Met Pro Leu Glu Asn Pro Lys Arg Glu Asp Gly Pro
370 375 380

Leu Ile Ile Leu His Gly Asn Leu Ala Pro Asp Gly Ala Val Ala Lys
385 390 395 400

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

Asn Ser Glu Glu Glu Ala Ile Glu Ala Val Leu Asn Asp Asp Ile Val
420 425 430

Asp Gly Asp Val Val Val Val Arg Phe Val Gly Pro Lys Gly Gly Pro
435 440 445

Gly Met Pro Glu Met Leu Ser Leu Ser Ser Met Ile Val Gly Lys Gly
450 455 460

Gln Gly Glu Lys Val Ala Leu Leu Thr Asp Gly Arg Phe Ser Gly Gly

[0119]

465 470 475 480

Thr Tyr Gly Leu Val Val Gly His Ile Ala Pro Glu Ala Gln Asp Gly
485 490 495

Gly Pro Ile Ala Tyr Leu Gln Thr Gly Asp Ile Val Thr Ile Asp Gln
500 505 510

Asp Thr Lys Glu Leu His Phe Asp Ile Ser Asp Glu Glu Leu Lys His
515 520 525

Arg Gln Glu Thr Ile Glu Leu Pro Pro Leu Tyr Ser Arg Gly Ile Leu
530 535 540

Gly Lys Tyr Ala His Ile Val Ser Ser Ala Ser Arg Gly Ala Val Thr
545 550 555 560

Asp Phe Trp Lys Pro Glu Glu Thr Gly Lys Lys
565 570

<210> 98
<211> 1758
<212> DNA
<213> 啤酒糖酵母 (Saccharomyces cerevisiae)

<400> 98
atgggcttgt taacgaaagt tgctacatct agacaattct ctacaacgag atgcgttgca 60

aagaagctca acaagtactc gtatatcatc actgaaccta agggccaagg tgcgtcccag 120

gccatgcttt atgccaccgg tttcaagaag gaagatttca agaagcctca agtcggggtt 180

ggttcctgtt ggtggtccgg taacccatgt aacatgcatc tattggactt gaataacaga 240

tgttctcaat ccattgaaaa agcgggtttg aaagctatgc agttcaacac catcggtgtt 300

tcagacggta tctctatggg tactaaaggt atgagatact cgttacaaag tagagaaatc 360

attgcagact cctttgaaac catcatgatg gcacaacact acgatgctaa catcgccatc 420

ccatcatgtg acaaaaacat gcccggtgtc atgatggcca tgggtagaca taacagacct 480

tccatcatgg tatatggtgg tactatcttg cccggtcatc caacatgtgg ttcttcgaag 540

atctctaaaa acatcgatat cgtctctgcg ttccaatcct acggtgaata tatttccaag 600

caattcactg aagaagaaag agaagatgtt gtggaacatg catgcccagg tcctggttct 660

tgtggtggta tgtatactgc caacacaatg gcttctgccg ctgaagtgct aggtttgacc 720

attccaaact cctcttcctt cccagccgtt tccaaggaga agttagctga gtgtgacaac 780

attggtgaat acatcaagaa gacaatggaa ttgggtattt tacctcgtga tatcctcaca 840

aaagaggctt ttgaaaacgc cattacttat gtcgttgcaa ccggtgggtc cactaatgct 900

gttttgcatt tggtggctgt tgctcactct gcgggtgtca agttgtcacc agatgatttc 960

caaagaatca gtgatactac accattgatc ggtgacttca aaccttctgg taaatacgtc 1020

[0120]

atggccgatt tgattaacgt tggtggtacc caatctgtga ttaagtatct atatgaaaac 1080

aacatgttgc acggtaacac aatgactgtt accggtgaca ctttggcaga acgtgcaaag 1140

aaagcaccaa gcctacctga aggacaagag attattaagc cactctccca cccaatcaag 1200

gccaacggtc acttgcaaat tctgtacggt tcattggcac caggtggagc tgtgggtaaa 1260

attaccggta aggaaggtac ttacttcaag ggtagagcac gtgtgttcga agaggaaggt 1320

gcctttattg aagccttgga aagaggtgaa atcaagaagg gtgaaaaaac cgttgttgtt 1380

atcagatatg aaggtccaag aggtgcacca ggtatgcctg aaatgctaaa gccttcctct 1440

gctctgatgg gttacggttt gggtaaagat gttgcattgt tgactgatgg tagattctct 1500

ggtggttctc acgggttctt aatcggccac attgttcccg aagccgctga aggtggtcct 1560

atcgggttgg tcagagacgg cgatgagatt atcattgatg ctgataataa caagattgac 1620

ctattagtct ctgataagga aatggctcaa cgtaaacaaa gttgggttgc acctccacct 1680

cgttacacaa gaggtactct atccaagtat gctaagttgg tttccaacgc ttccaacggt 1740

tgtgttttag atgcttga 1758

<210> 99
<211> 585
<212> PRT
<213> 啤酒糖酵母（Saccharomyces cerevisiae）

<400> 99

Met Gly Leu Leu Thr Lys Val Ala Thr Ser Arg Gln Phe Ser Thr Thr
1 5 10 15

Arg Cys Val Ala Lys Lys Leu Asn Lys Tyr Ser Tyr Ile Ile Thr Glu
20 25 30

Pro Lys Gly Gln Gly Ala Ser Gln Ala Met Leu Tyr Ala Thr Gly Phe
35 40 45

Lys Lys Glu Asp Phe Lys Lys Pro Gln Val Gly Val Gly Ser Cys Trp
50 55 60

Trp Ser Gly Asn Pro Cys Asn Met His Leu Leu Asp Leu Asn Asn Arg
65 70 75 80

Cys Ser Gln Ser Ile Glu Lys Ala Gly Leu Lys Ala Met Gln Phe Asn
85 90 95

Thr Ile Gly Val Ser Asp Gly Ile Ser Met Gly Thr Lys Gly Met Arg
100 105 110

Tyr Ser Leu Gln Ser Arg Glu Ile Ile Ala Asp Ser Phe Glu Thr Ile
115 120 125

[0121]

Met Met Ala Gln His Tyr Asp Ala Asn Ile Ala Ile Pro Ser Cys Asp
130 135 140

Lys Asn Met Pro Gly Val Met Met Ala Met Gly Arg His Asn Arg Pro
145 150 155 160

Ser Ile Met Val Tyr Gly Gly Thr Ile Leu Pro Gly His Pro Thr Cys
165 170 175

Gly Ser Ser Lys Ile Ser Lys Asn Ile Asp Ile Val Ser Ala Phe Gln
180 185 190

Ser Tyr Gly Glu Tyr Ile Ser Lys Gln Phe Thr Glu Glu Glu Arg Glu
195 200 205

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

Tyr Thr Ala Asn Thr Met Ala Ser Ala Ala Glu Val Leu Gly Leu Thr
225 230 235 240

Ile Pro Asn Ser Ser Ser Phe Pro Ala Val Ser Lys Glu Lys Leu Ala
245 250 255

Glu Cys Asp Asn Ile Gly Glu Tyr Ile Lys Lys Thr Met Glu Leu Gly
260 265 270

Ile Leu Pro Arg Asp Ile Leu Thr Lys Glu Ala Phe Glu Asn Ala Ile
275 280 285

Thr Tyr Val Val Ala Thr Gly Gly Ser Thr Asn Ala Val Leu His Leu
290 295 300

Val Ala Val Ala His Ser Ala Gly Val Lys Leu Ser Pro Asp Asp Phe
305 310 315 320

Gln Arg Ile Ser Asp Thr Thr Pro Leu Ile Gly Asp Phe Lys Pro Ser
325 330 335

Gly Lys Tyr Val Met Ala Asp Leu Ile Asn Val Gly Gly Thr Gln Ser
340 345 350

Val Ile Lys Tyr Leu Tyr Glu Asn Asn Met Leu His Gly Asn Thr Met
355 360 365

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

Leu Pro Glu Gly Gln Glu Ile Ile Lys Pro Leu Ser His Pro Ile Lys

[0122]

385 390 395 400

Ala Asn Gly His Leu Gln Ile Leu Tyr Gly Ser Leu Ala Pro Gly Gly
405 410 415

Ala Val Gly Lys Ile Thr Gly Lys Glu Gly Thr Tyr Phe Lys Gly Arg
420 425 430

Ala Arg Val Phe Glu Glu Glu Gly Ala Phe Ile Glu Ala Leu Glu Arg
435 440 445

Gly Glu Ile Lys Lys Gly Glu Lys Thr Val Val Val Ile Arg Tyr Glu
450 455 460

Gly Pro Arg Gly Ala Pro Gly Met Pro Glu Met Leu Lys Pro Ser Ser
465 470 475 480

Ala Leu Met Gly Tyr Gly Leu Gly Lys Asp Val Ala Leu Leu Thr Asp
485 490 495

Gly Arg Phe Ser Gly Gly Ser His Gly Phe Leu Ile Gly His Ile Val
500 505 510

Pro Glu Ala Ala Glu Gly Gly Pro Ile Gly Leu Val Arg Asp Gly Asp
515 520 525

Glu Ile Ile Ile Asp Ala Asp Asn Asn Lys Ile Asp Leu Leu Val Ser
530 535 540

Asp Lys Glu Met Ala Gln Arg Lys Gln Ser Trp Val Ala Pro Pro Pro
545 550 555 560

Arg Tyr Thr Arg Gly Thr Leu Ser Lys Tyr Ala Lys Leu Val Ser Asn
565 570 575

Ala Ser Asn Gly Cys Val Leu Asp Ala
580 585

<210> 100
<211> 1647
<212> DNA
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 100
atgtatacag taggagatta cctattagac cgattacacg agttaggaat tgaagaaatt 60

tttggagtcc ctggagacta taacttacaa tttttagatc aaattatttc ccacaaggat 120

atgaaatggg tcggaaatgc taatgaatta aatgcttcat atatggctga tggctatgct 180

cgtactaaaa aagctgccgc atttcttaca acctttggag taggtgaatt gagtgcagtt 240

aatggattag caggaagtta cgccgaaaat ttaccagtag tagaaatagt gggatcacct 300

[0123]

acatcaaaag ttcaaaatga aggaaaattt gttcatcata cgctggctga cggtgatttt 360

aaacacttta tgaaaatgca cgaacctgtt acagcagctc gaactttact gacagcagaa 420

aatgcaaccg ttgaaattga ccgagtactt tctgcactat taaaagaaag aaaacctgtc 480

tatatcaact taccagttga tgttgctgct gcaaaagcag agaaaccctc actccctttg 540

aaaaaggaaa actcaacttc aaatacaagt gaccaagaaa ttttgaacaa aattcaagaa 600

agcttgaaaa atgccaaaaa accaatcgtg attacaggac atgaaataat tagttttggc 660

ttagaaaaaa cagtcactca atttatttca aagacaaaac tacctattac gacattaaac 720

tttggtaaaa gttcagttga tgaagccctc ccttcatttt taggaatcta taatggtaca 780

ctctcagagc ctaatcttaa agaattcgtg gaatcagccg acttcatctt gatgcttgga 840

gttaaactca cagactcttc aacaggagcc ttcactcatc atttaaatga aaataaaatg 900

atttcactga atatagatga aggaaaaata tttaacgaaa gaatccaaaa ttttgatttt 960

gaatccctca tctcctctct cttagaccta agcgaaatag aatacaaagg aaaatatatc 1020

gataaaaagc aagaagactt tgttccatca aatgcgcttt tatcacaaga ccgcctatgg 1080

caagcagttg aaaacctaac tcaaagcaat gaaacaatcg ttgctgaaca agggacatca 1140

ttctttggcg cttcatcaat tttcttaaaa tcaaagagtc attttattgg tcaaccctta 1200

tggggatcaa ttggatatac attcccagca gcattaggaa gccaaattgc agataaagaa 1260

agcagacacc ttttatttat tggtgatggt tcacttcaac ttacagtgca agaattagga 1320

ttagcaatca gagaaaaaat taatccaatt tgctttatta tcaataatga tggttataca 1380

gtcgaaagag aaattcatgg accaaatcaa agctacaatg atattccaat gtggaattac 1440

tcaaaattac cagaatcgtt tggagcaaca gaagatcgag tagtctcaaa aatcgttaga 1500

actgaaaatg aatttgtgtc tgtcatgaaa gaagctcaag cagatccaaa tagaatgtac 1560

tggattgagt taattttggc aaaagaaggt gcaccaaaag tactgaaaaa aatgggcaaa 1620

ctatttgctg aacaaaataa atcataa 1647

<210> 101
<211> 548
<212> PRT
<213> 乳酸乳球菌 (Lactococcus lactis)

<400> 101

Met Tyr Thr Val Gly Asp Tyr Leu Leu Asp Arg Leu His Glu Leu Gly
1 5 10 15

Ile Glu Glu Ile Phe Gly Val Pro Gly Asp Tyr Asn Leu Gln Phe Leu
20 25 30

Asp Gln Ile Ile Ser His Lys Asp Met Lys Trp Val Gly Asn Ala Asn
35 40 45

[0124]

Glu Leu Asn Ala Ser Tyr Met Ala Asp Gly Tyr Ala Arg Thr Lys Lys
50 55 60

Ala Ala Ala Phe Leu Thr Thr Phe Gly Val Gly Glu Leu Ser Ala Val
65 70 75 80

Asn Gly Leu Ala Gly Ser Tyr Ala Glu Asn Leu Pro Val Val Glu Ile
85 90 95

Val Gly Ser Pro Thr Ser Lys Val Gln Asn Glu Gly Lys Phe Val His
100 105 110

His Thr Leu Ala Asp Gly Asp Phe Lys His Phe Met Lys Met His Glu
115 120 125

Pro Val Thr Ala Ala Arg Thr Leu Leu Thr Ala Glu Asn Ala Thr Val
130 135 140

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

Tyr Ile Asn Leu Pro Val Asp Val Ala Ala Ala Lys Ala Glu Lys Pro
165 170 175

Ser Leu Pro Leu Lys Lys Glu Asn Ser Thr Ser Asn Thr Ser Asp Gln
180 185 190

Glu Ile Leu Asn Lys Ile Gln Glu Ser Leu Lys Asn Ala Lys Lys Pro
195 200 205

Ile Val Ile Thr Gly His Glu Ile Ile Ser Phe Gly Leu Glu Lys Thr
210 215 220

Val Thr Gln Phe Ile Ser Lys Thr Lys Leu Pro Ile Thr Thr Leu Asn
225 230 235 240

Phe Gly Lys Ser Ser Val Asp Glu Ala Leu Pro Ser Phe Leu Gly Ile
245 250 255

Tyr Asn Gly Thr Leu Ser Glu Pro Asn Leu Lys Glu Phe Val Glu Ser
260 265 270

Ala Asp Phe Ile Leu Met Leu Gly Val Lys Leu Thr Asp Ser Ser Thr
275 280 285

Gly Ala Phe Thr His His Leu Asn Glu Asn Lys Met Ile Ser Leu Asn
290 295 300

Ile Asp Glu Gly Lys Ile Phe Asn Glu Arg Ile Gln Asn Phe Asp Phe

[0125]

305 310 315 320

Glu Ser Leu Ile Ser Ser Leu Leu Asp Leu Ser Glu Ile Glu Tyr Lys
325 330 335

Gly Lys Tyr Ile Asp Lys Lys Gln Glu Asp Phe Val Pro Ser Asn Ala
340 345 350

Leu Leu Ser Gln Asp Arg Leu Trp Gln Ala Val Glu Asn Leu Thr Gln
355 360 365

Ser Asn Glu Thr Ile Val Ala Glu Gln Gly Thr Ser Phe Phe Gly Ala
370 375 380

Ser Ser Ile Phe Leu Lys Ser Lys Ser His Phe Ile Gly Gln Pro Leu
385 390 395 400

Trp Gly Ser Ile Gly Tyr Thr Phe Pro Ala Ala Leu Gly Ser Gln Ile
405 410 415

Ala Asp Lys Glu Ser Arg His Leu Leu Phe Ile Gly Asp Gly Ser Leu
420 425 430

Gln Leu Thr Val Gln Glu Leu Gly Leu Ala Ile Arg Glu Lys Ile Asn
435 440 445

Pro Ile Cys Phe Ile Ile Asn Asn Asp Gly Tyr Thr Val Glu Arg Glu
450 455 460

Ile His Gly Pro Asn Gln Ser Tyr Asn Asp Ile Pro Met Trp Asn Tyr
465 470 475 480

Ser Lys Leu Pro Glu Ser Phe Gly Ala Thr Glu Asp Arg Val Val Ser
485 490 495

Lys Ile Val Arg Thr Glu Asn Glu Phe Val Ser Val Met Lys Glu Ala
500 505 510

Gln Ala Asp Pro Asn Arg Met Tyr Trp Ile Glu Leu Ile Leu Ala Lys
515 520 525

Glu Gly Ala Pro Lys Val Leu Lys Lys Met Gly Lys Leu Phe Ala Glu
530 535 540

Gln Asn Lys Ser
545

<210> 102
<211> 1644
<212> DNA

[0126]

<213> 人工序列

<220>
<223> 经密码子优化以在植物乳杆菌（Lactobacillus plantarum）中表达的编码区

<400> 102
atgtataccg ttggtgacta tttgttggat cggttacacg aattaggcat cgaagaaatc 60

ttcggtgttc caggcgatta caatttacaa tttttggacc aaatcatcag tcacaaagac 120

atgaaatggg tcggtaacgc aaatgaatta aatgcgagtt acatggctga cggttatgct 180

cggactaaga aagctgcagc ctttttgacg acttttggtg ttggtgaatt atcagccgtt 240

aatggtttgg ccggtagtta cgccgaaaat ttaccggtcg ttgaaattgt tggcagtcca 300

acgagtaagg ttcaaaacga aggtaaattt gtccatcata ccttggcaga tggtgatttt 360

aaacacttta tgaagatgca tgaaccggtc actgctgcac ggactttatt aactgcggaa 420

aatgccaccg tcgaaattga ccgcgtttta tcagccttgt taaaagaacg caaaccggtt 480

tacattaatt taccggtcga tgtggcagcg gccaaagctg aaaaaccgag tttgccgtta 540

aagaaggaaa atagtacctc aaatacctca gatcaagaaa tcttgaacaa gatccaagaa 600

tcattaaaaa atgctaaaaa accaatcgtt attaccggtc atgaaattat tagttttggt 660

ttggaaaaaa cggtgacgca atttatttca aagacgaaat taccaattac gaccttgaat 720

tttggtaaat caagtgttga cgaagcgttg ccaagtttct tgggtatcta taatggtact 780

ttaagtgaac cgaatttaaa agaatttgtg gaaagtgcag attttatttt aatgttgggt 840

gtcaagttaa ctgattcatc aactggcgcg ttcacgcatc atttaaatga aaataaaatg 900

atttcattga atattgatga aggcaaaatc ttcaacgaac ggattcaaaa ttttgatttt 960

gaatcattga ttagtagttt gttagattta tcagaaatcg aatacaaagg caagtatatt 1020

gataagaagc aggaagattt tgttccgagt aatgcattgt taagtcaaga tcgcttatgg 1080

caggcggttg aaaacttaac tcaatcaaac gaaaccattg ttgctgaaca aggtacttca 1140

ttcttcggcg catcaagtat ctttttaaaa tcaaaaagtc atttcatcgg tcaaccatta 1200

tggggtagta ttggttacac cttcccagcg gcgttaggta gtcaaattgc tgacaaagaa 1260

tcacgacact tattattcat tggtgacggt agtttacaat tgacggtcca ggaattaggt 1320

ttggccattc gcgaaaagat caacccaatt tgtttcatta tcaataatga cggttatact 1380

gttgaacggg aaattcacgg tccgaaccaa agttacaatg atattccaat gtggaactac 1440

tcaaaattgc cggaaagttt tggcgccacc gaagatcggg tcgttagtaa aattgtgcgg 1500

accgaaaatg aattcgtgtc agtgatgaag gaagcacaag ccgatccgaa ccggatgtat 1560

tggattgaat taatcttggc taaggaaggt gccccgaagg ttttaaagaa gatgggcaag 1620

ttatttgcag aacaaaataa aagt 1644

<210> 103
<211> 1047
<212> DNA

[0127]

<213> 木糖氧化无色杆菌（Achromobacter xylosoxidans）

<400> 103

atgaaagctc tggtttatca cggtgaccac aagatctcgc ttgaagacaa gcccaagccc 60

acccttcaaa agcccacgga tgtagtagta cgggttttga agaccacgat ctgcggcacg 120

gatctcggca tctacaaagg caagaatcca gaggtcgccg acgggcgcat cctgggccat 180

gaaggggtag gcgtcatcga ggaagtgggc gagagtgtca cgcagttcaa gaaaggcgac 240

aaggtcctga tttcctgcgt cacttcttgc ggctcgtgcg actactgcaa gaagcagctt 300

tactcccatt gccgcgacgg cgggtggatc ctgggttaca tgatcgatgg cgtgcaggcc 360

gaatacgtcc gcatcccgca tgccgacaac agcctctaca agatccccca gacaattgac 420

gacgaaatcg ccgtcctgct gagcgacatc ctgcccaccg gccacgaaat cggcgtccag 480

tatgggaatg tccagccggg cgatgcggtg gctattgtcg gcgcgggccc cgtcggcatg 540

tccgtactgt tgaccgccca gttctactcc ccctcgacca tcatcgtgat cgacatggac 600

gagaatcgcc tccagctcgc caaggagctc ggggcaacgc acaccatcaa ctccggcacg 660

gagaacgttg tcgaagccgt gcataggatt gcggcagagg gagtcgatgt tgcgatcgag 720

gcggtgggca taccggcgac ttgggacatc tgccaggaga tcgtcaagcc cggcgcgcac 780

atcgccaacg tcggcgtgca tggcgtcaag gttgacttcg agattcagaa gctctggatc 840

aagaacctga cgatcaccac gggactggtg aacacgaaca cgacgcccat gctgatgaag 900

gtcgcctcga ccgacaagct tccgttgaag aagatgatta cccatcgctt cgagctggcc 960

gagatcgagc acgcctatca ggtattcctc aatggcgcca aggagaaggc gatgaagatc 1020

atcctctcga acgcaggcgc tgcctga 1047

<210> 104
<211> 348
<212> PRT
<213> 木糖氧化无色杆菌（Achromobacter xylosoxidans）

<400> 104

Met Lys Ala Leu Val Tyr His Gly Asp His Lys Ile Ser Leu Glu Asp
1 5 10 15

Lys Pro Lys Pro Thr Leu Gln Lys Pro Thr Asp Val Val Val Arg Val
20 25 30

Leu Lys Thr Thr Ile Cys Gly Thr Asp Leu Gly Ile Tyr Lys Gly Lys
35 40 45

Asn Pro Glu Val Ala Asp Gly Arg Ile Leu Gly His Glu Gly Val Gly
50 55 60

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

[0128]

Lys Val Leu Ile Ser Cys Val Thr Ser Cys Gly Ser Cys Asp Tyr Cys
85 90 95

Lys Lys Gln Leu Tyr Ser His Cys Arg Asp Gly Gly Trp Ile Leu Gly
100 105 110

Tyr Met Ile Asp Gly Val Gln Ala Glu Tyr Val Arg Ile Pro His Ala
115 120 125

Asp Asn Ser Leu Tyr Lys Ile Pro Gln Thr Ile Asp Asp Glu Ile Ala
130 135 140

Val Leu Leu Ser Asp Ile Leu Pro Thr Gly His Glu Ile Gly Val Gln
145 150 155 160

Tyr Gly Asn Val Gln Pro Gly Asp Ala Val Ala Ile Val Gly Ala Gly
165 170 175

Pro Val Gly Met Ser Val Leu Leu Thr Ala Gln Phe Tyr Ser Pro Ser
180 185 190

Thr Ile Ile Val Ile Asp Met Asp Glu Asn Arg Leu Gln Leu Ala Lys
195 200 205

Glu Leu Gly Ala Thr His Thr Ile Asn Ser Gly Thr Glu Asn Val Val
210 215 220

Glu Ala Val His Arg Ile Ala Ala Glu Gly Val Asp Val Ala Ile Glu
225 230 235 240

Ala Val Gly Ile Pro Ala Thr Trp Asp Ile Cys Gln Glu Ile Val Lys
245 250 255

Pro Gly Ala His Ile Ala Asn Val Gly Val His Gly Val Lys Val Asp
260 265 270

Phe Glu Ile Gln Lys Leu Trp Ile Lys Asn Leu Thr Ile Thr Thr Gly
275 280 285

Leu Val Asn Thr Asn Thr Thr Pro Met Leu Met Lys Val Ala Ser Thr
290 295 300

Asp Lys Leu Pro Leu Lys Lys Met Ile Thr His Arg Phe Glu Leu Ala
305 310 315 320

Glu Ile Glu His Ala Tyr Gln Val Phe Leu Asn Gly Ala Lys Glu Lys
325 330 335

Ala Met Lys Ile Ile Leu Ser Asn Ala Gly Ala Ala

[0129]

340 345

<210> 105
<211> 1047
<212> DNA
<213> 人工序列

<220>
<223> 经密码子优化以在植物乳杆菌（Lactobacillus plantarum）中表达的sadB编码区

<400> 105
atgaaagctt tggtttacca tggtgaccac aaaattagtt tagaagataa gccaaaacca 60

actttgcaga agccaactga tgtcgtggtc cgtgtcttaa agacgaccat ttgtggcacg 120

gacttgggta tctataaggg caagaatcca gaagttgccg acggtcgtat cttaggtcat 180

gaaggcgtcg gtgttatcga agaagttggt gaaagtgtta cccaattcaa gaagggtgac 240

aaagttttaa tcagttgtgt tacgagttgt ggttcatgtg attactgtaa gaaacaattg 300

tacagtcatt gtcgtgacgg tggttggatc ttgggttaca tgattgatgg tgtccaagct 360

gaatacgtcc gtattccaca cgcagataat tcattgtata aaattccaca aactattgac 420

gacgaaattg ctgttttgtt atcagatatc ttaccaacgg gccatgaaat tggtgtccaa 480

tatggcaacg tccaaccagg cgatgccgtt gcaattgttg gcgccggtcc agtgggcatg 540

agtgttttat taacggctca attctattca ccatcaacta tcattgtcat cgacatggat 600

gaaaatcgtt tgcaattggc taaggaattg ggcgctacgc acactatcaa ttcaggtacg 660

gaaaatgttg ttgaagcagt ccatcgtatt gcagctgaag gcgtcgatgt ggcaattgaa 720

gccgtgggca ttccagcaac gtgggatatt tgtcaagaaa ttgttaagcc aggcgcccat 780

atcgccaatg tgggtgtgca tggtgtgaag gttgattttg aaattcaaaa attgtggatt 840

aaaaatttga cgattactac tggtttggtt aacacgaata cgactccaat gttaatgaag 900

gtcgcatcaa ccgataaatt accattgaag aagatgatta ctcaccgttt tgaattagca 960

gaaattgaac atgcctatca agttttctta aacggcgcca aggaaaaagc aatgaaaatt 1020

attttatcaa acgccggtgc agcttaa 1047

<210> 106
<211> 1125
<212> DNA
<213> 人工序列

<220>
<223> 经密码子优化以在啤酒糖酵母（S. cerevisiae）中表达的马ADH编码区

<400> 106
atgtcaacag ccggtaaagt tattaagtgt aaagcggcag ttttgtggga agagaaaaag 60

ccgtttagca tagaagaagt agaagtagcg ccaccaaaag cacacgaggt tagaatcaag 120

atggttgcca ccggaatctg tagatccgac gaccatgtgg tgagtggcac tctagttact 180

cctttgccag taatcgcggg acacgaggct gccggaatcg ttgaatccat aggtgaaggt 240

[0130]

gttaccactg ttcgtcctgg tgataaagtg atcccactgt tcactcctca atgtggtaag 300

tgtagagtct gcaaacatcc tgagggtaat ttctgcctta aaaatgattt gtctatgcct 360

agaggtacta tgcaggatgg tacaagcaga tttacatgca gagggaaacc tatacaccat 420

ttccttggta cttctacatt ttcccaatac acagtggtgg acgagatatc tgtcgctaaa 480

atcgatgcag cttcaccact ggaaaaagtt tgcttgatag ggtgcggatt ttccaccggt 540

tacggttccg cagttaaagt tgcaaaggtt acacagggtt cgacttgtgc agtattcggt 600

ttaggaggag taggactaag cgttattatg gggtgtaaag ctgcaggcgc agcgaggatt 660

ataggtgtag acatcaataa ggacaaattt gcaaaagcta aggaggtcgg ggctactgaa 720

tgtgttaacc ctcaagatta taagaaacca atacaagaag tccttactga aatgtcaaac 780

ggtggagttg atttctcttt tgaagttata ggccgtcttg atactatggt aactgcgttg 840

tcctgctgtc aagaggcata tggagtcagt gtgatcgtag gtgttcctcc tgattcacaa 900

aatttgtcga tgaatcctat gctgttgcta agcggtcgta catggaaggg agctatattt 960

ggcggtttta agagcaagga tagtgttcca aaacttgttg ccgactttat ggcgaagaag 1020

tttgctcttg atcctttaat tacacatgta ttgccattcg agaaaatcaa tgaagggttt 1080

gatttgttaa gaagtggtga atctattcgt acaattttaa ctttt 1125

<210> 107
<211> 375
<212> PRT
<213> 野马 (Equus caballus)

<400> 107

Met Ser Thr Ala Gly Lys Val Ile Lys Cys Lys Ala Ala Val Leu Trp
1 5 10 15

Glu Glu Lys Lys Pro Phe Ser Ile Glu Glu Val Glu Val Ala Pro Pro
20 25 30

Lys Ala His Glu Val Arg Ile Lys Met Val Ala Thr Gly Ile Cys Arg
35 40 45

Ser Asp Asp His Val Val Ser Gly Thr Leu Val Thr Pro Leu Pro Val
50 55 60

Ile Ala Gly His Glu Ala Ala Gly Ile Val Glu Ser Ile Gly Glu Gly
65 70 75 80

Val Thr Thr Val Arg Pro Gly Asp Lys Val Ile Pro Leu Phe Thr Pro
85 90 95

Gln Cys Gly Lys Cys Arg Val Cys Lys His Pro Glu Gly Asn Phe Cys
100 105 110

[0131]

Leu Lys Asn Asp Leu Ser Met Pro Arg Gly Thr Met Gln Asp Gly Thr
115 120 125

Ser Arg Phe Thr Cys Arg Gly Lys Pro Ile His His Phe Leu Gly Thr
130 135 140

Ser Thr Phe Ser Gln Tyr Thr Val Val Asp Glu Ile Ser Val Ala Lys
145 150 155 160

Ile Asp Ala Ala Ser Pro Leu Glu Lys Val Cys Leu Ile Gly Cys Gly
165 170 175

Phe Ser Thr Gly Tyr Gly Ser Ala Val Lys Val Ala Lys Val Thr Gln
180 185 190

Gly Ser Thr Cys Ala Val Phe Gly Leu Gly Gly Val Gly Leu Ser Val
195 200 205

Ile Met Gly Cys Lys Ala Ala Gly Ala Ala Arg Ile Ile Gly Val Asp
210 215 220

Ile Asn Lys Asp Lys Phe Ala Lys Ala Lys Glu Val Gly Ala Thr Glu
225 230 235 240

Cys Val Asn Pro Gln Asp Tyr Lys Lys Pro Ile Gln Glu Val Leu Thr
245 250 255

Glu Met Ser Asn Gly Gly Val Asp Phe Ser Phe Glu Val Ile Gly Arg
260 265 270

Leu Asp Thr Met Val Thr Ala Leu Ser Cys Cys Gln Glu Ala Tyr Gly
275 280 285

Val Ser Val Ile Val Gly Val Pro Pro Asp Ser Gln Asn Leu Ser Met
290 295 300

Asn Pro Met Leu Leu Leu Ser Gly Arg Thr Trp Lys Gly Ala Ile Phe
305 310 315 320

Gly Gly Phe Lys Ser Lys Asp Ser Val Pro Lys Leu Val Ala Asp Phe
325 330 335

Met Ala Lys Lys Phe Ala Leu Asp Pro Leu Ile Thr His Val Leu Pro
340 345 350

Phe Glu Lys Ile Asn Glu Gly Phe Asp Leu Leu Arg Ser Gly Glu Ser
355 360 365

Ile Arg Thr Ile Leu Thr Phe

131

[0132]

370 375

<210> 108
<211> 1440
<212> DNA
<213> 大肠杆菌 (Escherichia coli)

<400> 108
atggtaacgt tcatgataac ttctgctctt catcgtgcgg ccgactgggc taaatctgtg 60

ttctcttcgg cggcgctggg tgatcctcgc cgtactgccc gcttggttaa cgtcgccgcc 120

caattggcaa aatattctgg taaatcaata accatctcat cagagggtag tgaagccatg 180

caggaaggcg cttaccgatt tatccgcaat cccaacgttt ctgccgaggc gatcagaaag 240

gctggcgcca tgcaaacagt caagttggct caggagtttc ccgaactgct ggccattgag 300

gacaccacct ctttgagtta tcgccaccag gtcgccgaag agcttggcaa gctgggctct 360

attcaggata aatcccgcgg atggtgggtt cactccgttc tcttgctcga ggccaccaca 420

ttccgcaccg taggattact gcatcaggag tggtggatgc gcccggatga ccctgccgat 480

gcggatgaaa aggagagtgg caaatggctg gcagccgccg caactagccg gttacgcatg 540

ggcagcatga tgagcaacgt gattgcggtc tgtgaccgcg aagccgatat tcatgcttat 600

ctgcaggaca aactggcgca taacgagcgc ttcgtggtgc gctccaagca cccacgcaag 660

gacgtagagt ctgggttgta tctgtacgac catctgaaga accaaccgga gttgggtggc 720

tatcagatca gcattccgca aaagggcgtg gtggataaac gcggtaaacg taaaaatcga 780

ccagcccgca aggcgagctt gagcctgcgc agtgggcgca tcacgctaaa acaggggaat 840

atcacgctca acgcggtgct ggccgaggag attaacccgc ccaagggtga gaccccgttg 900

aaatggttgt tgctgaccag cgaaccggtc gagtcgctag cccaagcctt gcgcgtcatc 960

gacatttata cccatcgctg gcggatcgag gagttccata aggcatggaa aaccggagca 1020

ggagccgaga ggcaacgcat ggaggagccg gataatctgg agcggatggt ctcgatcctc 1080

tcgtttgttg cggtcaggct gttacagctc agagaaagct tcacgctgcc gcaagcactc 1140

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ctgaccccgg atgaatgtca gctactgggc tatctggaca agggaaaacg caagcgcaaa 1260

gagaaagcag gtagcttgca gtgggcttac atggcgatag ctagactggg cggttttatg 1320

gacagcaagc gaaccggaat tgccagctgg ggcgccctct gggaaggttg ggaagccctg 1380

caaagtaaac tggatggctt tcttgccgcc aaggatctga tggcgcaggg gatcaagatc 1440

<210> 109
<211> 480
<212> PRT
<213> 大肠杆菌 (Escherichia coli)

<400> 109

Met Val Thr Phe Met Ile Thr Ser Ala Leu His Arg Ala Ala Asp Trp

[0133]

1 5 10 15

Ala Lys Ser Val Phe Ser Ser Ala Ala Leu Gly Asp Pro Arg Arg Thr
20 25 30

Ala Arg Leu Val Asn Val Ala Ala Gln Leu Ala Lys Tyr Ser Gly Lys
35 40 45

Ser Ile Thr Ile Ser Ser Glu Gly Ser Glu Ala Met Gln Glu Gly Ala
50 55 60

Tyr Arg Phe Ile Arg Asn Pro Asn Val Ser Ala Glu Ala Ile Arg Lys
65 70 75 80

Ala Gly Ala Met Gln Thr Val Lys Leu Ala Gln Glu Phe Pro Glu Leu
85 90 95

Leu Ala Ile Glu Asp Thr Thr Ser Leu Ser Tyr Arg His Gln Val Ala
100 105 110

Glu Glu Leu Gly Lys Leu Gly Ser Ile Gln Asp Lys Ser Arg Gly Trp
115 120 125

Trp Val His Ser Val Leu Leu Leu Glu Ala Thr Thr Phe Arg Thr Val
130 135 140

Gly Leu Leu His Gln Glu Trp Trp Met Arg Pro Asp Asp Pro Ala Asp
145 150 155 160

Ala Asp Glu Lys Glu Ser Gly Lys Trp Leu Ala Ala Ala Ala Thr Ser
165 170 175

Arg Leu Arg Met Gly Ser Met Met Ser Asn Val Ile Ala Val Cys Asp
180 185 190

Arg Glu Ala Asp Ile His Ala Tyr Leu Gln Asp Lys Leu Ala His Asn
195 200 205

Glu Arg Phe Val Val Arg Ser Lys His Pro Arg Lys Asp Val Glu Ser
210 215 220

Gly Leu Tyr Leu Tyr Asp His Leu Lys Asn Gln Pro Glu Leu Gly Gly
225 230 235 240

Tyr Gln Ile Ser Ile Pro Gln Lys Gly Val Val Asp Lys Arg Gly Lys
245 250 255

Arg Lys Asn Arg Pro Ala Arg Lys Ala Ser Leu Ser Leu Arg Ser Gly
260 265 270

[0134]

Arg Ile Thr Leu Lys Gln Gly Asn Ile Thr Leu Asn Ala Val Leu Ala
275 280 285

Glu Glu Ile Asn Pro Pro Lys Gly Glu Thr Pro Leu Lys Trp Leu Leu
290 295 300

Leu Thr Ser Glu Pro Val Glu Ser Leu Ala Gln Ala Leu Arg Val Ile
305 310 315 320

Asp Ile Tyr Thr His Arg Trp Arg Ile Glu Glu Phe His Lys Ala Trp
325 330 335

Lys Thr Gly Ala Gly Ala Glu Arg Gln Arg Met Glu Glu Pro Asp Asn
340 345 350

Leu Glu Arg Met Val Ser Ile Leu Ser Phe Val Ala Val Arg Leu Leu
355 360 365

Gln Leu Arg Glu Ser Phe Thr Leu Pro Gln Ala Leu Arg Ala Gln Gly
370 375 380

Leu Leu Lys Glu Ala Glu His Val Glu Ser Gln Ser Ala Glu Thr Val
385 390 395 400

Leu Thr Pro Asp Glu Cys Gln Leu Leu Gly Tyr Leu Asp Lys Gly Lys
405 410 415

Arg Lys Arg Lys Glu Lys Ala Gly Ser Leu Gln Trp Ala Tyr Met Ala
420 425 430

Ile Ala Arg Leu Gly Gly Phe Met Asp Ser Lys Arg Thr Gly Ile Ala
435 440 445

Ser Trp Gly Ala Leu Trp Glu Gly Trp Glu Ala Leu Gln Ser Lys Leu
450 455 460

Asp Gly Phe Leu Ala Ala Lys Asp Leu Met Ala Gln Gly Ile Lys Ile
465 470 475 480

<210> 110
<211> 5323
<212> DNA
<213> 人工序列

<220>
<223> 载体

<400> 110
gaattcagat ctctcgagcc cgggatcgat ggtacctcgc gaaagcttgg atgttgtaca 60

ggataatgtc cagaaggtcg atagaaagcg tgagaaacag cgtacagacg atttagagat 120

[0135]

gtagaggtac ttttatgccg agaaaacttt ttgcgtgtga cagtccttaa aatatactta 180

gagcgtaagc gaaagtagta gcgacagcta ttaactttcg gttgcaaagc tctaggattt 240

ttaatggacg cagcgcatca cacgcaaaaa ggaaattgga ataaatgcga aatttgagat 300

gttaattaaa gacctttttg aggtcttttt ttcttagatt tttggggtta tttaggggag 360

aaaacatagg ggggtactac gacctccccc ctaggtgtcc attgtccatt gtccaaacaa 420

ataaataaat attgggtttt taatgttaaa aggttgtttt ttatgttaaa gtgaaaaaaa 480

cagatgttgg gaggtacagt gatagttgta gatagaaaag aagagaaaaa agttgctgtt 540

actttaagac ttacaacaga agaaaatgag atattaaata gaatcaaaga aaaatataat 600

attagcaaat cagatgcaac cggtattcta ataaaaaaat atgcaaagga ggaatacggt 660

gcattttaaa caaaaaaaga tagacagcac tggcatgctg cctatctatg actaaatttt 720

gttaagtgta ttagcaccgt tattatatca tgagcgaaaa tgtaataaaa gaaactgaaa 780

acaagaaaaa ttcaagagga cgtaattgga catttgtttt atatccagaa tcagcaaaag 840

ccgagtggtt agagtattta aaagagttac acattcaatt tgtagtgtct ccattacatg 900

atagggatac tgatacagaa ggtaggatga aaaaagagca ttatcatatt ctagtgatgt 960

atgagggtaa taaatcttat gaacagataa aaataattaa cagaagaatt gaatgcgact 1020

attccgcaga ttgcaggaag tgtgaaaggt cttgtgagat atatgcttca catggacgat 1080

cctaataaat ttaaatatca aaaagaagat atgatagttt atggcggtgt agatgttgat 1140

gaattattaa agaaaacaac aacagataga tataaattaa ttaaagaaat gattgagttt 1200

attgatgaac aaggaatcgt agaatttaag agtttaatgg attatgcaat gaagtttaaa 1260

tttgatgatt ggttcccgct tttatgtgat aactcggcgt atgttattca agaatatata 1320

aaatcaaatc ggtataaatc tgaccgatag attttgaatt taggtgtcac aagacactct 1380

tttttcgcac cagcgaaaac tggtttaagc cgactgcgca aaagacataa tcgattcaca 1440

aaaaataggc acacgaaaaa caagttaagg gatgcagttt atgcatccct taacttactt 1500

attaaataat ttatagctat tgaaaagaga taagaattgt tcaaagctaa tattgtttaa 1560

atcgtcaatt cctgcatgtt ttaaggaatt gttaaattga ttttttgtaa atattttctt 1620

gtattctttg ttaacccatt tcataacgaa ataattatac ttttgtttat ctttgtgtga 1680

tattcttgat ttttttctac ttaatctgat aagtgagcta ttcactttag gtttaggatg 1740

aaaatattct cttggaacca tacttaatat agaaatatca acttctgcca ttaaaagtaa 1800

tgccaatgag cgttttgtat ttaataatct tttagcaaac ccgtattcca cgattaaata 1860

aatctcatta gctatactat caaaaacaat tttgcgtatt atatccgtac ttatgttata 1920

aggtatatta ccatatattt tataggattg gtttttagga aatttaaact gcaatatatc 1980

cttgtttaaa acttggaaat tatcgtgatc aacaagttta ttttctgtag ttttgcataa 2040

tttatggtct atttcaatgg cagttacgaa attacacctc tttactaatt caagggtaaa 2100

[0136]

atggcctttt cctgagccga tttcaaagat attatcatgt tcatttaatc ttatatttgt 2160

cattatttta tctatattat gttttgaagt aataaagttt tgactgtgtt ttatattttt 2220

ctcgttcatt ataaccctct ttaatttggt tatatgaatt ttgcttatta acgattcatt 2280

ataaccactt atttttttgtt tggttgataa tgaactgtgc tgattacaaa aatactaaaa 2340

atgcccatat tttttcctcc ttataaaatt agtataatta tagcacgagc tctgataaat 2400

atgaacatga tgagtgatcg ttaaatttat actgcaatcg gatgcgatta ttgaataaaa 2460

gatatgagag atttatctaa tttctttttt cttgtaaaaa aagaaagttc ttaaaggttt 2520

tatagttttg gtcgtagagc acacggttta acgacttaat tacgaagtaa ataagtctag 2580

tgtgttagac tttatgaaat ctatatacgt ttatatatat ttattatccg gatctgcatc 2640

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aaaacctctg acacatgcag ctcccggaga cggtcacagc ttgtctgtaa gcggatgccg 3120

ggagcagaca agcccgtcag ggcgcgtcag cgggtgttgg cgggtgtcgg ggcgcagcca 3180

tgacccagtc acgtagcgat agcggagtgt atactggctt aactatgcgg catcagagca 3240

gattgtactg agagtgcacc atatgcggtg tgaaataccg cacagatgcg taaggagaaa 3300

ataccgcatc aggcgctctt ccgcttcctc gctcactgac tcgctgcgct cggtcgttcg 3360

gctgcggcga gcggtatcag ctcactcaaa ggcggtaata cggttatcca cagaatcagg 3420

ggataacgca ggaaagaaca tgtgagcaaa aggccagcaa aaggccagga accgtaaaaa 3480

ggccgcgttg ctggcgtttt tccataggct ccgcccccct gacgagcatc acaaaaatcg 3540

acgctcaagt cagaggtggc gaaacccgac aggactataa agataccagg cgtttccccc 3600

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actggcagca gccactggta acaggattag cagagcgagg tatgtaggcg gtgctacaga 3900

gttcttgaag tggtggccta actacggcta cactagaagg acagtatttg gtatctgcgc 3960

tctgctgaag ccagttacct tcggaaaaag agttggtagc tcttgatccg gcaaacaaac 4020

caccgctggt agcggtggtt tttttgtttg caagcagcag attacgcgca gaaaaaaagg 4080

[0137]

atctcaagaa gatcctttga tcttttctac ggggtctgac gctcagtgga acgaaaactc 4140

acgttaaggg attttggtca tgagattatc aaaaaggatc ttcacctaga tccttttaaa 4200

ttaaaaatga agttttaaat caatctaaag tatatatgag taaacttggt ctgacagtta 4260

ccaatgctta atcagtgagg cacctatctc agcgatctgt ctatttcgtt catccatagt 4320

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tgttgccatt gctgcaggca tcgtggtgtc acgctcgtcg tttggtatgg cttcattcag 4620

ctccggttcc caacgatcaa ggcgagttac atgatccccc atgttgtgca aaaaagcggt 4680

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ggttatggca gcactgcata attctcttac tgtcatgcca tccgtaagat gcttttctgt 4800

gactggtgag tactcaacca agtcattctg agaatagtgt atgcggcgac cgagttgctc 4860

ttgcccggcg tcaacacggg ataataccgc gccacatagc agaactttaa aagtgctcat 4920

cattggaaaa cgttcttcgg ggcgaaaact ctcaaggatc ttaccgctgt tgagatccag 4980

ttcgatgtaa cccactcgtg cacccaactg atcttcagca tcttttactt tcaccagcgt 5040

ttctgggtga gcaaaaacag gaaggcaaaa tgccgcaaaa aagggaataa gggcgacacg 5100

gaaatgttga tactcatac tcttcctttt tcaatattat tgaagcattt atcagggtta 5160

ttgtctcatg agcggataca tatttgaatg tatttagaaa aataaacaaa taggggttcc 5220

gcgcacattt ccccgaaaag tgccacctga cgtctaagaa accattatta tcatgacatt 5280

aacctataaa aataggcgta tcacgaggcc ctttcgtctt caa 5323

<210> 111
<211> 31
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 111
catgaattcg tgctaagagc cagattgtgg a 31

<210> 112
<211> 50
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 112
catgaagacc acgcgtaggc cttctagagc taaattttca catcgtgagc 50

[0138]

<210> 113
<211> 49
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 113
atttagctct agaaggccta cgcgtggtct tcatgaactt gttcaaccg 49

<210> 114
<211> 31
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 114
catctcgagc caagctcagt cacgcattta a 31

<210> 115
<211> 22
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 115
aagcacaacg ggaagcgaac at 22

<210> 116
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 116
atacaactat gacgctggaa gcg 23

<210> 117
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 117
gtaggttttc ccgtccttga tag 23

<210> 118
<211> 35
<212> DNA
<213> 人工序列

[0139]

<220>
<223> 引物

<400> 118
tataagatct tgactctggt gaacttgtcg caacc 35

<210> 119
<211> 34
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 119
atatctcgag aataagtcat cctctcgtag tgaa 34

<210> 120
<211> 34
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 120
tatactcgag taatcatttc atacgattaa atgt 34

<210> 121
<211> 32
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 121
atatcccggg gtgagcgggt aaagtccttg cc 32

<210> 122
<211> 710
<212> DNA
<213> 植物乳杆菌 (Lactobacillus plantarum)

<400> 122
atgaagcgac caattatcat tgcgttagat tttcccaccg ccgaacgggc cttagctttt 60

ttagaccaat ttccggctga tttacatgtc actgtcaaaa tcggcatgga gttattttat 120

gcagcgggac cgagtattgt gacggacgtg caagctcgcg gccatgcggt tttcttagat 180

ttgaaactac atgatattcc caataccgtc gaatccgcaa tgcgggtgat cgggcggtta 240

ggggtaacct atacgacggt tcatgctgcg ggtgggcacg tgatgctttc agccgccaaa 300

cgaggattgg tcgcgggtgc aatggccgct ggagtcactg cccccaagtt attagcgatt 360

acgcagttaa cttcgactaa tcaagctatt ttgaatcagg accagcaaat catgggaacg 420

gttcgggcga gtgtcgtgca ttatgccaaa ctagcacggg cgagtgactg tgatggcgtc 480

atttgttccg cccaagaagt tcaggcgatt catacggccg tcggtgctga ttttctcgga 540

[0140]

attacgccgg gaattcggcc agcgtcggcg cagtcagatg accagcaacg ggtgatgaca 600

ccggctgccg ctgctaaggc tgggagcaac ggtctcgtca tcgggcggcc aattacgcag 660

gctgcagaac cagttcaagc ttaccgagat attatgacag aatggagtaa 710

<210> 123
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 123
cgggcacctg caaccgaggt c 21

<210> 124
<211> 22
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 124
ctgtttctca cgctttctat cg 22

<210> 125
<211> 22
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 125
gattttcttt atcaacttcg ac 22

<210> 126
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 126
ttggaaaacg ttcttcgggg c 21

<210> 127
<211> 20
<212> DNA
<213> 人工序列

<220>
<223> 核糖体结合位点

<400> 127
atataggagg aatttttgta 20

[0141]

<210> 128
<211> 5124
<212> DNA
<213> 人工序列

<220>
<223> 构建质粒

<400> 128
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agatctatat tatagatctt gggggttatt tgttttaata ttaaagaaat gacttcttct 120

atttgtcatc aatactaaac aataatttgt acaaagtgat tatttcttct agttcttcac 180

gcgatacatg atcgacaata gtttcatcag tgacatgtct tgcccgtaaa tctaaggcta 240

tggtttgatc taataatact tttccatata ctgtttgact actagttagt cgatgataca 300

ttggaaaatt acgcttggta ctgctaattg gagccacaat cgtcatgtta cttgtctgac 360

agactagatc attgcttagc gcaatggctg gtcgcttatt catctgttca tgaccacggc 420

ttggattaaa gttaacataa aatatatcac cttggcttac cattgaagtt cattaccttc 480

tgactttccc caatcaagct cgtgatccct tttcccgtca tctttccaat ccttaaatag 540

ttcgtgaata ttggttgggt tctttttttat tggtgttaaa acaattgatc cattttcaat 600

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tctagcagct ttcgagtttc cccactttgc taagcgtgtt tgttctttaa taagttccat 720

attttcccct cctaaattat tattacaagt caagtatatc ccatgtagat acacaatgca 780

aatattctta ctggagaaat aacaccttaa gtctagcacc acccgcacgc atagcggtgc 840

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caccgtcatc accgaaacgc gcga 5124

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<211> 3748
<212> DNA
<213> 人工序列

<220>
<223> 载体

<400> 129
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agatctatat tatagatctt gggggttatt tgttttaata ttaaagaaat gacttcttct 120

atttgtcatc aatactaaac aataatttgt acaaagtgat tatttcttct agttcttcac 180

[0144]

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ttggaaaatt acgcttggta ctgctaattg gagccacaat cgtcatgtta cttgtctgac 360

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tctagcagct ttcgagtttc cccactttgc taagcgtgtt tgttctttaa taagttccat 720

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aatattctta ctggagaaat aacaccttaa gtctagcacc acccgcacgc atagcggtgc 840

ttaaaccatc aagggtcaag cccttaggct ctctcaaaca gttatcctaa tcgtgaataa 900

ctgcgcttct tttgcagtat aaagagagaa ctctttatca gacaatttaa gctcaaccag 960

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gacaaggttt agcataccta tgttgttaac tgcaagcggg gtcacgaacg acactcacgg 1140

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aatcctaact ctgctaagtt gagggttctt ttttttgcgt tcatttatta agttgagtac 1440

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[0145]

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ttttcaccgt catcaccgaa acgcgcga 3748

<210> 130
<211> 22
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 130
ttgtctcgag gaccgagcgc ag 22

[0146]

<210> 131
<211> 60
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 131
tatagacaag cccgtcctgc agttaagctt ttggatcctg ttatccgctc acaattccac 60

<210> 132
<211> 1730
<212> DNA
<213> 人工序列

<220>
<223> 具有核糖体结合位点和来自乳酸乳球菌（L. lactis）的ilvD编码区的PCR片段

<400> 132
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tcaataaata ttctaagaca ttgactcaag atccaacaca accagcgact caagcgatgt 120

actacggcat tggttttaaa gatgaggatt tcaaaaaagc tcaggtcgga atcgtcagca 180

tggattggga cggaaatcca tgtaatatgc acttgggaac acttgggagt aaaatcaaaa 240

gttctgtcaa ccaaactgac ggattgattg gacttcaatt tcatactatt ggagtttctg 300

atggaattgc taacggaaag cttggcatga gatattcttt ggtcagtcgt gaagttattg 360

ctgacagcat cgaaaccaac gctggcgcag aatattatga tgccatcgtt gccattcccg 420

gttgtgataa aaatatgccc gggtcaatta tcggaatggc tcgcttaaat cgtccgtcaa 480

ttatggtcta tggtggaacg attgaacatg gcgaatataa aggtgaaaaa ttaaatattg 540

tttcggcctt tgaagctctg gggcaaaaaa tcactggaaa tatttctgat gaagattatc 600

atggcgttat ttgcaatgcc attccaggac aaggtgcttg cggaggaatg tacactgcca 660

ataccctggc tgctgctatt gaaactttgg gaatgagttt accttattcc tcttccaatc 720

cagcagtcag tcaagaaaaa caagaagagt gtgatgacat tggtttagcc atcaaaaatt 780

tattagaaaa agatattaaa ccaagtgata tcatgaccaa agaagctttt gaaaatgcca 840

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tgaccgtcac aggtaaaact ttggctgaaa atgttgaaac agcattagat ttggactttg 1140

acagtcaaga tattatgcga ccactaaaaa atccaattaa agctactgga catttacaaa 1200

ttttgtacgg taatcttgcc caaggggttt ctgttgcaaa aatttctggt aaagaaggcg 1260

aatttttcaa aggaacagct cgtgtttttg acggagaaca acactttatc gatggcattg 1320

[0147]

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gtccgggaat gccagagatg ttaaaaccaa cctcagcatt aattggagca ggacttggaa 1440

aatcttgtgc cctaattact gacggaagat tttctggtgg cacacacggc tttgttgtgg 1500

gtcatatcgt ccctgaagca gttgaaggtg ggttgattgg tttagttgaa gatgatgata 1560

ttatcgaaat tgatgcggtg aataatagta ttagtttaaa agtttctaat gaagaaattg 1620

ctaaacgacg tgccaattat caaaaaccaa cccctaaagc aacgcgtggt gttcttgcaa 1680

aatttgccaa acttacgcgc cccgctagtg aaggttgcgt tacagattta 1730

<210> 133
<211> 33
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 133
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<210> 134
<211> 31
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 134
atatctcgag ctataaatct gtaacgcaac c 31

<210> 135
<211> 1014
<212> DNA
<213> 人工序列

<220>
<223> PCR片段：添加到植物乳杆菌（L. plantarum）的suf操纵子5’部分的限制性位点

<400> 135
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tctcgtgaga ttttaaaagg cgtcaattta tctatgaaaa ctggtgaaat tcatgccatt 120

atgggaccaa atgggactgg taagtccact ttatcacaaa ctattatggg ccaaccggct 180

tatcacgtta ctcagggtga tatcttgttg aacggcgaaa gtatcgtaaa catgccagtt 240

gatgaacgtg cacgtaaggg actcttcctc ggcatgcagt atccagctga aattcaaggg 300

gtcaccaacg ctgaattttt acgggcagca atgaacgcac gccgaccagc cgatgatcaa 360

atctcagtga tggcctttct taaagaactc gacaagaact tggcactact taatatgagc 420

gaatccatga cggaacgtta cctaaacgaa ggtttctccg gtggtgaaaa gaagcgtaac 480

gaaattttgc aattattgat gatcaagcca tcattcgcct tattggacga aattgattcc 540

[0148]

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aatttcggct cattgattat cacgcattat caacggctgt taaactacat tgtgcccgat 660

gtcgttcacg tgatgatggg tggtcgaatc gtgaagactg gtaacgccga cttagcaaag 720

acccttgaaa aagaaggtta tgctggttta cgtgacgatt tgaacattga tgtcaaactt 780

gttgacgacg aagattaggg ggtggcagta atggaagcaa ctgctgatta tgaaactatc 840

aaaacaacgc tagctgcggc tgctaacgaa catggtgaac cgcactggct cgttgaacgc 900

cgtttagcgg ctttagatgc gatgcaaggc ctagcggttc ccaaagctga tcgctttagt 960

attcgcgact ggccactgac gcccaccgac caaccactaa agttcagtcg ttcg 1014

<210> 136
<211> 57
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 136
tagctagccc gggatatata ggaggaattt ttgtaatggc aaccttggaa gttaaag 57

<210> 137
<211> 39
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 137
attatgtcag gtacccgaac gactgaactt tagtggttg 39

<210> 138
<211> 93
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 138
tgtaggactc gagagatctc ggccgggagt tgttgacaca caaaaccaga catggtatta 60

taatctataa gcgagatctg ctcggggagt tgt 93

<210> 139
<211> 74
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 139
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[0149]

ccccgagcag atct 74

<210> 140
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 140
tgtaggactc gagagatctc g 21

<210> 141
<211> 16
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 141
tagctagccc ggggcg 16

<210> 142
<211> 1331
<212> DNA
<213> 人工序列

<220>
<223> PCR产物；限制性位点和sufP

<400> 142
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cgacgtggct ggtgcttgcc aagttttgac tagcgctggc taaggtttgc tcaataaagt 120

ctagtcgggt ttggaaaatc tgctcgtcga atttttgagc gtcgtaataa gcggcttgac 180

tagtgagtgg tgtcaatccc atgtgtttcg cgtaggctcg aattggctta ttttgattaa 240

taaattgaat cgtcagccac cgtgcgaagt ctgctgtcaa atcaaatttt ttgatgagct 300

gttggctagc ctgacgaatg gcttgctcaa tcatgaaagg atagttgagt ttgagcggtg 360

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ccgtcgtcat cttaatggcc tccaatcaac gtttgaaata cggtgtaccg aatcccaatc 900

[0150]

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agcggactgc cgacatgtag ccctaagctg tgcaatcgtt gaacggtctg attatccaaa 1020

cctgtgaatt gttgaatatg tagttgtgat gtattagtaa cggattgact tagcataagg 1080

ctcagtcctt tctgaatatt ccaatagtga ataattcatc aaaaatatta tacctacatc 1140

atagcatgaa agggctttta ttaatatggg gaaaagcctt attttctttt gaaatgaaaa 1200

cggttatact ctaagtatag aatacaaaaa ggccgatgct acgctatttt tgttgaagcc 1260

gttgactaat cattatatac ggaaggaata acggcttggg taataaattc aattgttgga 1320

ggatgattta a 1331

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<220>
<223> 引物

<400> 143
tgctgattga attccgaaga cgggtagccc taag 34

<210> 144
<211> 37
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<220>
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tgtaggactc gagttaaatc atcctccaac aattgaa 37

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<212> DNA
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<220>
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<400> 145
gcatcaagcg gtccgtaact ag 22

<210> 146
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<220>
<223> 引物

<400> 146
gcgctatacc gagattatat catgc 25

[0151]

<210> 147
<211> 28
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<220>
<223> 引物

<400> 147
ccagacatgg tattataatc tataagcg 28

<210> 148
<211> 22
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 148
tggtttatca atcccgcgac tc 22

<210> 149
<211> 6249
<212> DNA
<213> 人工序列

<220>
<223> 构建质粒

<400> 149
cgaaagcttg tctaacacac tagacttatt tacttcgtaa ttaagtcgtt aaaccgtgtg 60

ctctacgacc aaaactataa aaccttlaag aactttcttt ttttacaaga aaaaagaaat 120

tagataaatc tctcatatct tttattcaat aatcgcatcc gattgcagta taaatttaac 180

gatcactcat catgttcata tttatcagag ctcgtgctat aattatacta attttataag 240

gaggaaaaaa tatgggcatt tttagtattt ttgtaatcag cacagttcat tatcaaccaa 300

acaaaaaata agtggttata atgaatcgtt aataagcaaa attcatataa ccaaattaaa 360

gagggttata atgaacgaga aaaatataaa acacagtcaa aactttatta cttcaaaaca 420

taatatagat aaaataatga caaatataag attaaatgaa catgataata tctttgaaat 480

cggctcagga aaaggccatt ttacccttga attagtaaag aggtgtaatt tcgtaactgc 540

cattgaaata gaccataaat tatgcaaaac tacagaaaat aaacttgttg atcacgataa 600

tttccaagtt ttaaacaagg atatattgca gtttaaattt cctaaaaacc aatcctataa 660

aatatatggt aatatacctt ataacataag tacggatata atacgcaaaa ttgtttttga 720

tagtatagct aatgagattt atttaatcgt ggaatacggg tttgctaaaa gattattaaa 780

tacaaaacgc tcattggcat tacttttaat ggcagaagtt gatatttcta tattaagtat 840

ggttccaaga gaatattttc atcctaaacc taaagtgaat agctcactta tcagattaag 900

tagaaaaaaa tcaagaatat cacacaaaga taaacaaaag tataattatt tcgttatgaa 960

atgggttaac aaagaataca agaaaatatt tacaaaaaat caatttaaca attccttaaa 1020

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tgctgttact ttaagactta caacagaaga aaatgagata ttaaatagaa tcaaagaaaa 1680

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actgaaaaca agaaaaattc aagaggacgt aattggacat ttgttttata tccagaatca 1920

gcaaaagccg agtggttaga gtatttaaaa gagttacaca ttcaatttgt agtgtctcca 1980

ttacatgata gggatactga tacagaaggt aggatgaaaa aagagcatta tcatattcta 2040

gtgatgtatg agggtaataa atcttatgaa cagataaaaa taattaacag aagaattgaa 2100

tgcgactatt ccgcagattg caggaagtgt gaaaggtctt gtgagatata tgcttcacat 2160

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gcttcccaac cttcccagag ggcgccccag ctggcaattc cggttcgctt gctgtccata 2700

aaaccgccca gtctagctat cgccatgtaa gcccactgca agctacctgc tttctctttg 2760

cgcttgcgtt ttcccttgtc cagatagccc agtagctgac attcatccgg ggtcagcacc 2820

gtttctgcgg actggctttc tacgtgttcc gcttcttta gcagcccttg cgccctgagt 2880

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[0153]

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gcagggtcat ccgggcgcat ccaccactcc tgatgcagta atcctacggt gcggaatgtg 3600

gtggcctcga gcaagagaac ggagtgaacc caccatccgc gggatttatc ctgaatagag 3660

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gcgacgttaa ccaagcgggc agtacggcga ggatcaccca gcgccgccga agagaacaca 3960

gatttagccc agtcggccgc acgatgaaga gcagaagtta tcatgaacgt taccataata 4020

aatccccctt tttgaaaata atgaagactt atattgttat aataaaccag caatctcgca 4080

ttctgcaata taaaactaga ctccgcggct gaattgatag aattgtccca tgcgggctgt 4140

cggcgggcgg tgtcagggga taagccgaga gacacgtgtt ggattagaca tgagtcgaat 4200

gacgcgattt tttctggaaa aaatgacaaa tgaagacggg aaaaatgata ggggaaaatg 4260

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taaacgataa cttcgtataa tgtatgctat acgaagttat tctagattat aaaagccagt 4380

cattaggcct atctgacaat tcctgaatag agttcataaa caatcctgca tgataaccat 4440

cacaaacaga atgatgtacc tgtaaagata gcggtaaata tattgaatta cctttattaa 4500

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taaacccagt aaatgaagtc catggaataa tagaaagaga aaaagcattt tcaggtatag 4620

gtgttttggg aaacaatttc cccgaaccat tatatttctc tacatcagaa aggtataaat 4680

cataaaactc tttgaagtca ttctttacag gagtccaaat accagagaat gttttagata 4740

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tggccattgc ggccgcatag gatccatttg tcgactactt gtgtataaga gtcaggcgct 5340

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tctgacgctc aaatcagtgg tggcgaaacc cgacaggact ataaagatac caggcgtttc 5700

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cgctgttatg gccgcgtttg tctcattcca cgcctgacac tcagttccgg gtaggcagtt 5820

cgctccaagc tggactgtat gcacgaaccc cccgttcagt ccgaccgctg cgccttatcc 5880

ggtaactatc gtcttgagtc caacccggaa agacatgcaa aagcaccact ggcagcagcc 5940

actggtaatt gatttagagg agttagtctt gaagtcatgc gccggttaag gctaaactga 6000

aaggacaagt tttggtgact gcgctcctcc aagccagtta cctcggttca aagagttggt 6060

agctcagaga accttcgaaa aaccgccctg caaggcggtt ttttcgtttt cagagcaaga 6120

gattacgcgc agaccaaaac gatctcaaga agatcatctt attaatcaga taaaatattt 6180

ctagatttca gtgcaattta tctcttcaaa tgtagcacct gaagtcagcc ccatacgata 6240

taagttgtg 6249

<210> 150
<211> 979
<212> DNA
<213> 人工序列

<220>
<223> 合成片段：包含Tn5IE，loxP，氯霉素抗性基因（cm）和loxP的Tn5IE-loxP-cm-loxP盒

<400> 150
ctgtctcttg atcagatctc acaccgtgaa cgcgttgctt aagtgtttaa acgataactt 60

cgtataatgt atgctatacg aagttattct agattataaa agccagtcat taggcctatc 120

tgacaattcc tgaatagagt tcataaacaa tcctgcatga taaccatcac aaacagaatg 180

atgtacctgt aaagatagcg gtaaatatat tgaattacct ttattaatga attttcctgc 240

tgtaataatg ggtagaaggt aattactatt attattgata tttaagttaa acccagtaaa 300

tgaagtccat ggaataatag aaagagaaaa agcattttca ggtataggtg ttttgggaaa 360

[0155]

caatttcccc gaaccattat atttctctac atcagaaagg tataaatcat aaaactcttt 420

gaagtcattc tttacaggag tccaaatacc agagaatgtt ttagatacac catcaaaaat 480

tgtataaagt ggctctaact tatcccaata acctaactct ccgtcgctat tgtaaccagt 540

tctaaaagct gtatttgagt ttatcaccct tgtcactaag aaaataaatg cagggtaaaa 600

tttatatcct tcttgtttta tgtttcggta taaaacacta atatcaattt ctgtggttat 660

actaaaagtc gtttgttggt tcaaataatg attaaatatc tcttttctct tccaattgtc 720

taaatcaatt ttattaaagt tcatttgata tgcctcctaa ataattgtga gcgctcacaa 780

ttccacacat tatgccacac cttgtagata aagtcaacaa ctttttgcaa aatttttcag 840

gaattttagc agaggttgtt ctggatgtag aacaaaacat ctttccgctc ttgtgctgtt 900

aggatatctt tcttggaagc taggtaggca agggctacct ctagaataac ttcgtataat 960

gtatgctata cgaagttat 979

<210> 151
<211> 57
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 151
cgatatttgt cgactacttg tgtataagag tcaggcgcta gcggagtgta tactggc 57

<210> 152
<211> 42
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 152
ctttatacga cgtcacaact tatatcgtat ggggctgact tc 42

<210> 153
<211> 41
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 153
ttatactaag cttgtctaac acactagact tatttacttc g 41

<210> 154
<211> 42
<212> DNA
<213> 人工序列

<220>

[0156]

<223> 引物

<400> 154
ctctagtaag cttcatcgat gaactgcatc ccttaactta ct 42

<210> 155
<211> 251
<212> DNA
<213> 人工序列

<220>
<223> 启动子pPnpr

<400> 155
gatctaacat ttcccctat catttttccc gtcttcattt gtcatttttt ccagaaaaaa 60

tcgcgtcatt cgactcatgt ctaatccaac acgtgtctct cggcttatcc cctgacaccg 120

cccgccgaca gcccgcatgg gacaattcta tcaattcagc cgcggagtct agttttatat 180

tgcagaatgc gagattgctg gtttattata acaatataag tcttcattat tttcaaaaag 240

ggggatttat t 251

<210> 156
<211> 47
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 156
ggattacgat gcatgatcta acattttccc ctatcatttt tcccgtc 47

<210> 157
<211> 49
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 157
atcatgaacg ttaccataat aaatcccccc ttttgaaaat aatgaaaac 49

<210> 158
<211> 49
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 158
tttcaaaaag ggggatttat tatggtaacg ttcatgataa cttctgctc 49

<210> 159
<211> 46
<212> DNA
<213> 人工序列

[0157]

<220>
<223> 引物

<400> 159
actcatctta gatgcatcag atcttgatcc cctgcgccat cagatc 46

<210> 160
<211> 1694
<212> DNA
<213> 人工序列

<220>
<223> 具有Pnrp和thp编码区的PCR片段

<400> 160
gatctaacat tttcccctat catttttccc gtcttcattt gtcatttttt ccagaaaaaa 60

tcgcgtcatt cgactcatgt ctaatccaac acgtgtctct cggcttatcc cctgacaccg 120

cccgccgaca gcccgcatgg gacaattcta tcaattcagc cgcggagtct agttttatat 180

tgcagaatgc gagattgctg gtttattata acaatataag tcttcattat tttcaaaaag 240

ggggatttat tatggtaacg ttcatgataa cttctgctct tcatcgtgcg gccgactggg 300

ctaaatctgt gttctcttcg gcggcgctgg gtgatcctcg ccgtactgcc cgcttggtta 360

acgtcgccgc ccaattggca aaatattctg gtaaatcaat aaccatctca tcagagggta 420

gtgaagccat gcaggaaggc gcttaccgat ttatccgcaa tcccaacgtt tctgccgagg 480

cgatcagaaa ggctggcgcc atgcaaacag tcaagttggc tcaggagttt cccgaactgc 540

tggccattga ggacaccacc tctttgagtt atcgccacca ggtcgccgaa gagcttggca 600

agctgggctc tattcaggat aaatcccgcg gatggtgggt tcactccgtt ctcttgctcg 660

aggccaccac attccgcacc gtaggattac tgcatcagga gtggtggatg cgcccggatg 720

accctgccga tgcggatgaa aaggagagtg gcaaatggct ggcagccgcc gcaactagcc 780

ggttacgcat gggcagcatg atgagcaacg tgattgcggt ctgtgaccgc gaagccgata 840

ttcatgctta tctgcaggac aaactggcgc ataacgagcg cttcgtggtg cgctccaagc 900

acccacgcaa ggacgtagag tctgggttgt atctgtacga ccatctgaag aaccaaccgg 960

agttgggtgg ctatcagatc agcattccgc aaaagggcgt ggtggataaa cgcggtaaac 1020

gtaaaaatcg accagcccgc aaggcgagct tgagcctgcg cagtgggcgc atcacgctaa 1080

aacaggggaa tatcacgctc aacgcggtgc tggccgagga gattaacccg cccaagggtg 1140

agaccccgtt gaaatggttg ttgctgacca gcgaaccggt cgagtcgcta gcccaagcct 1200

tgcgcgtcat cgacatttat acccatcgct ggcggatcga ggagttccat aaggcatgga 1260

aaaccggagc aggagccgag aggcaacgca tggaggagcc ggataatctg gagcggatgg 1320

tctcgatcct ctcgtttgtt gcggtcaggc tgttacagct cagagaaagc ttcacgctgc 1380

cgcaagcact cagggcgcaa gggctgctaa aggaagcgga acacgtagaa agccagtccg 1440

cagaaacggt gctgaccccg gatgaatgtc agctactggg ctatctggac aagggaaaac 1500

[0158]

gcaagcgcaa agagaaagca ggtagcttgc agtgggctta catggcgata gctagactgg 1560

gcggttttat ggacagcaag cgaaccggaa ttgccagctg gggcgccctc tgggaaggtt 1620

gggaagccct gcaaagtaaa ctggatggct ttcttgccgc caaggatctg atggcgcagg 1680

ggatcaagat ctga 1694

<210> 161
<211> 29
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 161
ccaccacgcc cttttgcgga atgctgatc 29

<210> 162
<211> 29
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 162
cggccgcacg atgaagagca gaagttatc 29

<210> 163
<211> 29
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 163
ctgaccgata gattttgaat ttaggtgtc 29

<210> 164
<211> 114
<212> DNA
<213> 人工序列

<220>
<223> 启动子片段PgroE

<400> 164
aatgatgtaa gcgtgaaaaa ttttttatct tatcacttga aattggaagg gagattcttt 60

attataagaa ttgtggaatt gtgagcggat aacaattccc aattaaagga ggaa 114

<210> 165
<211> 42
<212> DNA
<213> 人工序列

<220>

[0159]

<223> 引物

<400> 165
acattgtcga cggtaccgct aacggaaaag ggagcggaaa ag 42

<210> 166
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 166
gacctccccg gatccttcct cctttaa 27

<210> 167
<211> 1681
<212> DNA
<213> 人工序列

<220>
<223> 具有核糖体结合位点和来自乳酸乳球菌（L. plantarum）的KivD编码区的PCR片段

<400> 167
ggatccgttt aaacataaaa tatggaggaa tgcgatgtat accgttggtg actatttgtt 60

ggatcggtta cacgaattag gcatcgaaga aatcttcggt gttccaggcg attacaattt 120

acaatttttg gaccaaatca tcagtcacaa agacatgaaa tgggtcggta acgcaaatga 180

attaaatgcg agttacatgg ctgacggtta tgctcggact aagaaagctg cagccttttt 240

gacgactttt ggtgttggtg aattatcagc cgttaatggt ttggccggta gttacgccga 300

aaatttaccg gtcgttgaaa ttgttggcag tccaacgagt aaggttcaaa acgaaggtaa 360

atttgtccat cataccttgg cagatggtga ttttaaacac tttatgaaga tgcatgaacc 420

ggtcactgct gcacggactt tattaactgc ggaaaatgcc accgtcgaaa ttgaccgcgt 480

tttatcagcc ttgttaaaag aacgcaaacc ggtttacatt aatttaccgg tcgatgtggc 540

agcggccaaa gctgaaaaac cgagtttgcc gttaaagaag gaaaatagta cctcaaatac 600

ctcagatcaa gaaatcttga acaagatcca agaatcatta aaaaatgcta aaaaaccaat 660

cgttattacc ggtcatgaaa ttattagttt tggtttggaa aaaacggtga cgcaatttat 720

ttcaaagacg aaattaccaa ttacgacctt gaattttggt aaatcaagtg ttgacgaagc 780

gttgccaagt ttcttgggta tctataatgg tactttaagt gaaccgaatt taaaagaatt 840

tgtggaaagt gcagatttta ttttaatgtt gggtgtcaag ttaactgatt catcaactgg 900

cgcgttcacg catcatttaa atgaaaataa aatgatttca ttgaatattg atgaaggcaa 960

aatcttcaac gaacggattc aaaattttga ttttgaatca ttgattagta gtttgttaga 1020

tttatcagaa atcgaataca aaggcaagta tattgataag aagcaggaag attttgttcc 1080

gagtaatgca ttgttaagtc aagatcgctt atggcaggcg gttgaaaact taactcaatc 1140

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[0160]

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<223> 引物

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<210> 169
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<220>
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[0162]

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atgatgacct cgtttccacc ggaattagct tggtaccagc tattgtaaca taatcggtac 4620

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agcggataac aattcccaat taaaggagga aggatccaaa agcttaactg caggacgggc 4800

[0168]

ttgtctgctc ccggcatccg cttacagaca agctgtgacc gtctccggga gctgcatgtg 4860

tcagaggttt tcaccgtcat caccgaaacg cgcga 4895

<210> 176
<211> 1548
<212> DNA
<213> 人工序列

<220>
<223> 具有融合到lacO操纵子序列和lacI阻遏蛋白基因上的枯草芽孢杆菌（B. subtilis）groE启动子（PgroE）的PCR片段

<400> 176
ccgagcaggc cttaactcac attaattgcg ttgcgctcac tgcccgcttt ccagtcggga 60

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attgggcgcc agggtggttt ttcttttcac cagtgagacg ggcaacagct gattgccctt 180

caccgcctgg ccctgagaga gttgcagcaa gcggtccacg ctggtttgcc ccagcaggcg 240

aaaatcctgt ttgatggtgg ttgacggcgg gatataacat gagctgtctt cggtatcgtc 300

gtatcccact accgagatat ccgcaccaac gcgcagcccg gactcggtaa tggcgcgcat 360

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cagcatttgc atggtttgtt gaaaaccgga catggcactc cagtcgcctt cccgttccgc 480

tatcggctga atttgattgc gagtgagata tttatgccag ccagccagac gcagacgcgc 540

cgagacagaa cttaatgggc ccgctaacag cgcgatttgc tggtgaccca atgcgaccag 600

atgctccacg cccagtcgcg taccgtcttc atgggagaaa ataatactgt tgatgggtgt 660

ctggtcagag acatcaagaa ataacgccgg aacattagtg caggcagctt ccacagcaat 720

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attgtgcacc gccgctttac aggcttcgac gccgcttcgt tctaccatcg acaccaccac 840

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cagggccaga ctggaggtgg caacgccaat cagcaacgac tgtttgcccg ccagttgttg 960

tgccacgcgg ttgggaatgt aattcagctc cgccatcgcc gcttccactt tttcccgcgt 1020

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ggcatactct gcgacatcgt ataacgttac tggtttcatc aaaatcgtct ccctccgttt 1140

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ataatcggta cgggggtgaa aaagctaacg gaaaagggag cggaaaagaa tgatgtaagc 1440

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[0169]

gtggaattgt gagcggataa caattcccaa ttaaaggagg aaggatcc 1548

<210> 177
<211> 32
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 177
gcgttacaga tttataggcg gccgctaagt cg 32

<210> 178
<211> 49
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 178
aatacattgt aactgccatt acaaaaattc ctcctatata tctcgaggc 49

<210> 179
<211> 51
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 179
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<210> 180
<211> 47
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 180
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<210> 181
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<220>
<223> 引物

<400> 181
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<210> 182
<211> 6207
<212> DNA

[0170]

<213> 人工序列

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<223> 构建质粒

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atttgtcatc aatactaaac aataatttgt acaaagtgat tatttcttct agttcttcac 180

gcgatacatg atcgacaata gtttcatcag tgacatgtct tgcccgtaaa tctaaggcta 240

tggtttgatc taataatact tttccatata ctgtttgact actagttagt cgatgataca 300

ttggaaaatt acgcttggta ctgctaattg gagccacaat cgtcatgtta cttgtctgac 360

agactagatc attgcttagc gcaatggctg gtcgcttatt catctgttca tgaccacggc 420

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tgactttccc caatcaagct cgtgatccct tttcccgtca tctttccaat ccttaaatag 540

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ggttattgtc atatcttggt tatcatctaa tttcagttgt ttaataattt ggctaggaat 660

tctagcagct ttcgagtttc cccactttgc taagcgtgtt tgttctttaa taagttccat 720

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gacaaggttt agcataccta tgttgttaac tgcaagcggg gtcacgaacg acactcacgg 1140

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[0171]

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[0172]

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<210> 183
<211> 1039
<212> DNA
<213> 人工序列

<220>
<223> 经优化以在植物乳杆菌（Lactobacillus plantarum）中表达的PF5-ilvC编码序列

<400> 183
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tattcaaggt aagaaggtcg ctattattgg ttatggttca caaggtcacg cccaagcctg 120

taatttgaag gatagtggtg ttgatgttac ggttggttta cggaagggta gtgctacggt 180

tgcgaaagcc gaagcgcatg gtttgaaggt tacggatgtt gcagctgccg tcgccggtgc 240

ggatttggtt atgattttaa cgccagatga atttcaaagt caattatata aaaatgaaat 300

tgaaccaaat attaaaaaag gtgctacgtt agcttttagt catggttttg cgattcatta 360

taatcaagtt gtcccacggg ctgatttaga tgtcattatg attgcaccaa aagctccagg 420

tcatacggtt cgtagtgaat ttgtcaaggg tggtggtatt ccagatttga ttgcaattta 480

tcaagatgca agtggtaatg ccaaaaatgt cgctttaagt tatgcggccg gtgttggtgg 540

tggtcggacg ggtattattg aaacgacgtt taaagatgaa acggaaacgg atttgtttgg 600

tgaacaagcc gtcttatgtg gtggtacggt tgaattagtt aaggcgggtt ttgaaacgtt 660

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aattgttgat ttgatgtatg aaggtggtat tgcaaatatg aattatagta ttagtaataa 780

tgcagaatat ggtgaatatg ttacgggtcc agaagttatt aatgcagaaa gtcggcaagc 840

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aggtgcgacg ggttatccaa gtatgacggc taagcggcgg aataatgccg cccatggtat 960

tgaaattatt ggtgaacaat tacggagtat gatgccatgg attggtgcta ataaaattgt 1020

tgataaggca aagaattaa 1039

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<211> 5581
<212> DNA

[0174]

<213> 人工序列

<220>
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<400> 184
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ccgccagtca agtgttcaaa agttagcgtt tattaagtgc gataagtata ccacaaaggg 120

cttattgacg cccgccaaag ggttttgcgg acattgttaa taattgtatt aaaagcatgc 180

tcaatctaac acttattttg cacaaacatg gtatacttta accgtaaaaa ctaaattttc 240

actacgagag gatgacttat tttgtcaagc ctcgagcccg ggatcgatgg tacctcgcga 300

aagcttggat gttgtacagg ataatgtcca gaaggtcgat agaaagcgtg agaaacagcg 360

tacagacgat ttagagatgt agaggtactt ttatgccgag aaaacttttt gcgtgtgaca 420

gtccttaaaa tatacttaga gcgtaagcga aagtagtagc gacagctatt aactttcggt 480

tgcaaagctc taggattttt aatggacgca gcgcatcaca cgcaaaaagg aaattggaat 540

aaatgcgaaa tttgagatgt taattaaaga ccttttttgag gtctttttttt cttagatttt 600

tggggttatt taggggagaa aacatagggg ggtactacga cctccccccct aggtgtccat 660

tgtccattgt ccaaacaaat aaataaatat tgggttttta atgttaaaag gttgtttttt 720

atgttaaagt gaaaaaaaca gatgttggga ggtacagtga tagttgtaga tagaaaagaa 780

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gcaaaggagg aatacggtgc attttaaaca aaaaaagata gacagcactg gcatgctgcc 960

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tatgcaatga agtttaaatt tgatgattgg ttcccgcttt tatgtgataa ctcggcgtat 1560

gttattcaag aatatataaa atcaaatcgg tataaatctg accgatagat tttgaattta 1620

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[0175]

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tactaattca agggtaaaat ggccttttcc tgagccgatt tcaaagatat tatcatgttc 2400

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gcacgagctc tgataaatat gaacatgatg agtgatcgtt aaatttatac tgcaatcgga 2700

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gctggtcgcg ctgttgtcaa aatgctcgaa atgggcttaa aaccttctga cattttaacg 780

cgtgaagctt ttgaagatgc tattactgta actatggctc tgggaggttc aaccaactca 840

acccttcacc tcttagctat tgcccatgct gctaatgtgg aattgacact tgatgatttc 900

aatactttcc aagaaaaagt tcctcatttg gctgatttga aaccttctgg tcaatatgta 960

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gtttctggtg taaaagtgcg tcgtcatgtc ggtcctgcta aggtctttaa ttctgaagaa 1260

gaagccattg aagctgtctt gaatgatgat attgttgatg gtgatgttgt tgtcgtacgt 1320

tttgtaggac caaagggcgg tcctggtatg cctgaaatgc tttccctttc atcaatgatt 1380

gttggtaaag ggcaaggtga aaaagttgcc cttctgacag atggccgctt ctcaggtggt 1440

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taatggaata ttatttttat ttatttattt atattattgg tcggctcttt tcttctgaag 1860

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ggccagtcca ctgcggagtc atttcaaagt catcctaatc gatctatcgt ttttgatagc 2040

tcattttgga gttcgcgatt gtcttctgtt attcacaact gttttaattt ttatttcatt 2100

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<220>
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tcaaagtatg actgacaaaa aaactcttaa agacttaag 99

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aatatatttc tccatac 77

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<212> DNA
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<400> 226

[0230]

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caccttggct aactcgttgt atcatcac 88

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<212> DNA
~~<213> 人工序列~~

<220>
<223> 引物

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<210> 229
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<220>
<223> 引物

<400> 229
cttggcagca acaggactag 20

<210> 230
<211> 26
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 230
ccaggccaat tcaacagact gtcggc 26

<210> 231
<211> 2347
<212> DNA
<213> 人工序列

<220>
<223> 具有用于HIS基因置换和标记切除的侧接同源重复序列的构建标记

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gcccagggtg aagatccacg ctactttacc ctgcgtcgtc tggatttcgg cggctgtcgt 120

[0231]

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cgtatcgcca cctcttatcc tcacctgctc aagcgttatc tcgaccagaa aggcatctct 240

tttaaatcct gcttactgaa cggttctgtt gaagtcgccc cgcgtgccgg actggcggat 300

gcgatttgcg atctggtttc caccggtgcc acgctggaag ctaacggcct gcgcgaagtc 360

gaagttatct atcgctcgaa agcctgcctg attcaacgcg atggcgaaat ggaagaatcc 420

aaacagcaac tgatcgacaa actgctgacc cgtattcagg gtgtgatcca ggcgcgcgaa 480

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aaggaaggag cacagactta gattggtata tatacgcata tgtagtgttg aagaaacatg 720

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attatccgcc aagtacaatt ttttactctt cgaagacaga aaatttgctg acattggtaa 1080

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cgacaaactg ctgacccgta ttcagggtgt gatccaggcg cgcgaatcaa aatacatcat 2280

gatgcacgca ccgaccgaac gtctggatga agtcatccag tgatgataca acgagttagc 2340

caaggtg 2347

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ttacgtattc taatgttcag 80

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<211> 80
<212> DNA
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<220>
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[0233]

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<220>
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<400> 237
ctgcagcgag gagccgtaat 20

<210> 238
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gcattgcgga ttacgtattc taatgttcag 90

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<211> 91
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<213> 人工序列

<220>
<223> 引物

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caccttggct aactcgttgt atcatcactg g 91

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<220>
<223> 引物

<400> 240
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<210> 241
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[0234]

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<400> 241
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<210> 242
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<220>
<223> 引物

<400> 242
gtgaacgagt tcacaaccgc 20

<210> 243
<211> 22
<212> DNA
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<220>
<223> 引物

<400> 243
gttcgttcca gaattatcac gc 22

<210> 244
<211> 1002
<212> DNA
<213> 人葡萄球菌（Staphylococcus hominis）

<400> 244
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cctgatgaaa ttcaaggtaa tgtatataaa aatgaaattg ctccaaattt agaagctggt 300

aatgcattag cttttgcaca tggatttaat attcatttta acgtcatcga accacctaaa 360

gatgtagatg tcttcttagt agcacctaaa ggtcctggtc atctagtaag acgtacattt 420

gttgaaggtt cagctgtgcc agcattattt ggtgttcaac aagatgctac aggtcatgca 480

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acaacattta aagaggaaac tgaaactgac ttattcggtg aacaagctgt actttgtggg 600

ggaattcata aactgattca aagtggtttc gagacattag tggaagctgg atatcaaaaa 660

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ggcggtatgg aaaatgtacg ttattcaatt tcaaatactg ctgaatttgg tgactacgta 780

tctggtccac gtgtcattac tccagatgtt aaagacaata tgaaagaagt acttaaagat 840

attcaaaatg gtaatttcgc taatcgcttt gttaaagata acgaaaatgg atttaaagaa 900

[0235]

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agaaaaatga tgccattcat taaatctaaa agcattcaaa aa 1002

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<213> 人葡萄球菌（Staphylococcus hominis）

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1 5 10 15

Gly Lys Lys Ile Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala His
20 25 30

Ala Gln Asn Leu Lys Asp Asn Gly Tyr Asp Val Arg Val Gly Ile Arg
35 40 45

Pro Gly Asp Ser Phe Asn Lys Ala Lys Glu Asp Gly Leu Asp Val Tyr
50 55 60

Pro Val Ala Glu Ala Val Lys Gln Ala Asp Val Ile Met Val Leu Leu
65 70 75 80

Pro Asp Glu Ile Gln Gly Asn Val Tyr Lys Asn Glu Ile Ala Pro Asn
85 90 95

Leu Glu Ala Gly Asn Ala Leu Ala Phe Ala His Gly Phe Asn Ile His
100 105 110

Phe Asn Val Ile Glu Pro Pro Lys Asp Val Asp Val Phe Leu Val Ala
115 120 125

Pro Lys Gly Pro Gly His Leu Val Arg Arg Thr Phe Val Glu Gly Ser
130 135 140

Ala Val Pro Ala Leu Phe Gly Val Gln Gln Asp Ala Thr Gly His Ala
145 150 155 160

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

Gly Val Ile Glu Thr Thr Phe Lys Glu Glu Thr Glu Thr Asp Leu Phe
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Ile His Lys Leu Ile Gln Ser
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Gln Lys Glu Leu Ala Tyr

[0236]

210 215 220

Phe Glu Val Leu His Glu Met Lys Leu Ile Val Asp Leu Met Tyr Glu
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245 250 255

Gly Asp Tyr Val Ser Gly Pro Arg Val Ile Thr Pro Asp Val Lys Asp
260 265 270

Asn Met Lys Glu Val Leu Lys Asp Ile Gln Asn Gly Asn Phe Ala Asn
275 280 285

Arg Phe Val Lys Asp Asn Glu Asn Gly Phe Lys Glu Phe Tyr Glu Leu
~~290~~ ~~295~~ ~~300~~

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Arg Lys Met Met Pro Phe Ile Lys Ser Lys Ser Ile Gln Lys
325 330

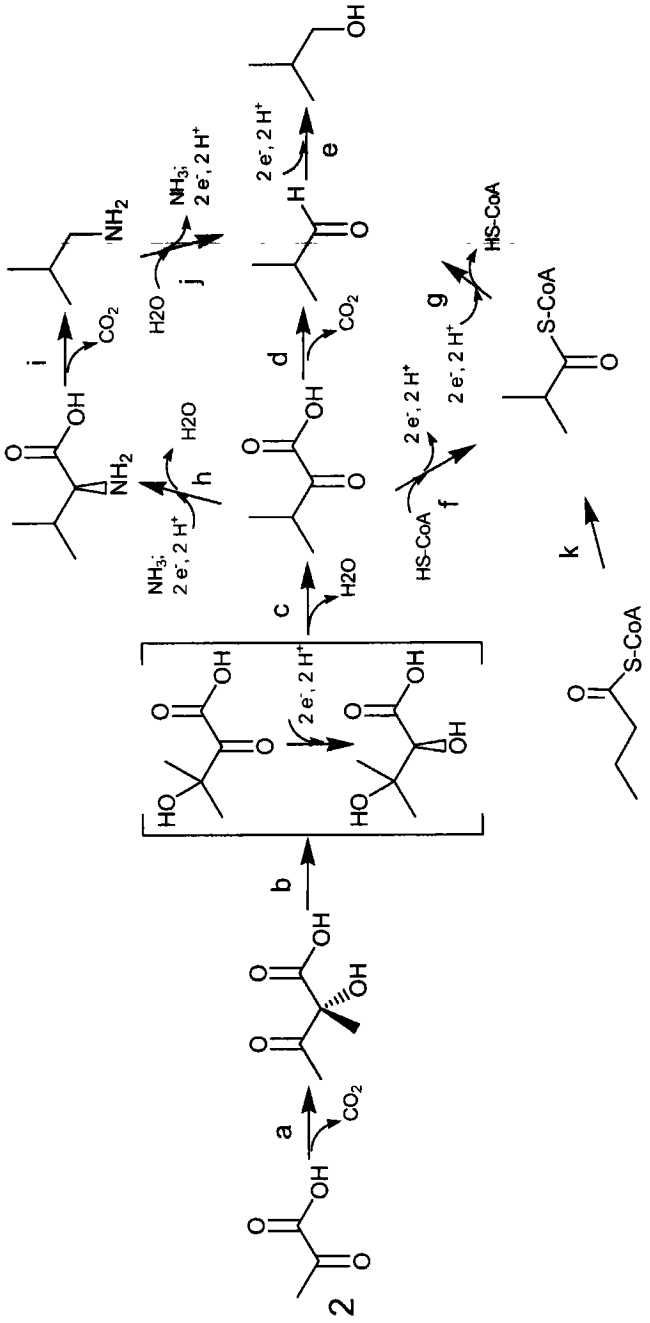

2
a
CO2
b
c
H2O
2e-, 2H+
d
CO2
e
2e-, 2H+
f
2e-, 2H+
HS-CoA
g
2e-, 2H+
HS-CoA
h
H2O
NH3;
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i
CO2
j
H2O
NH3;
2e-, 2H+
k
S-CoA
S-CoA

图 1

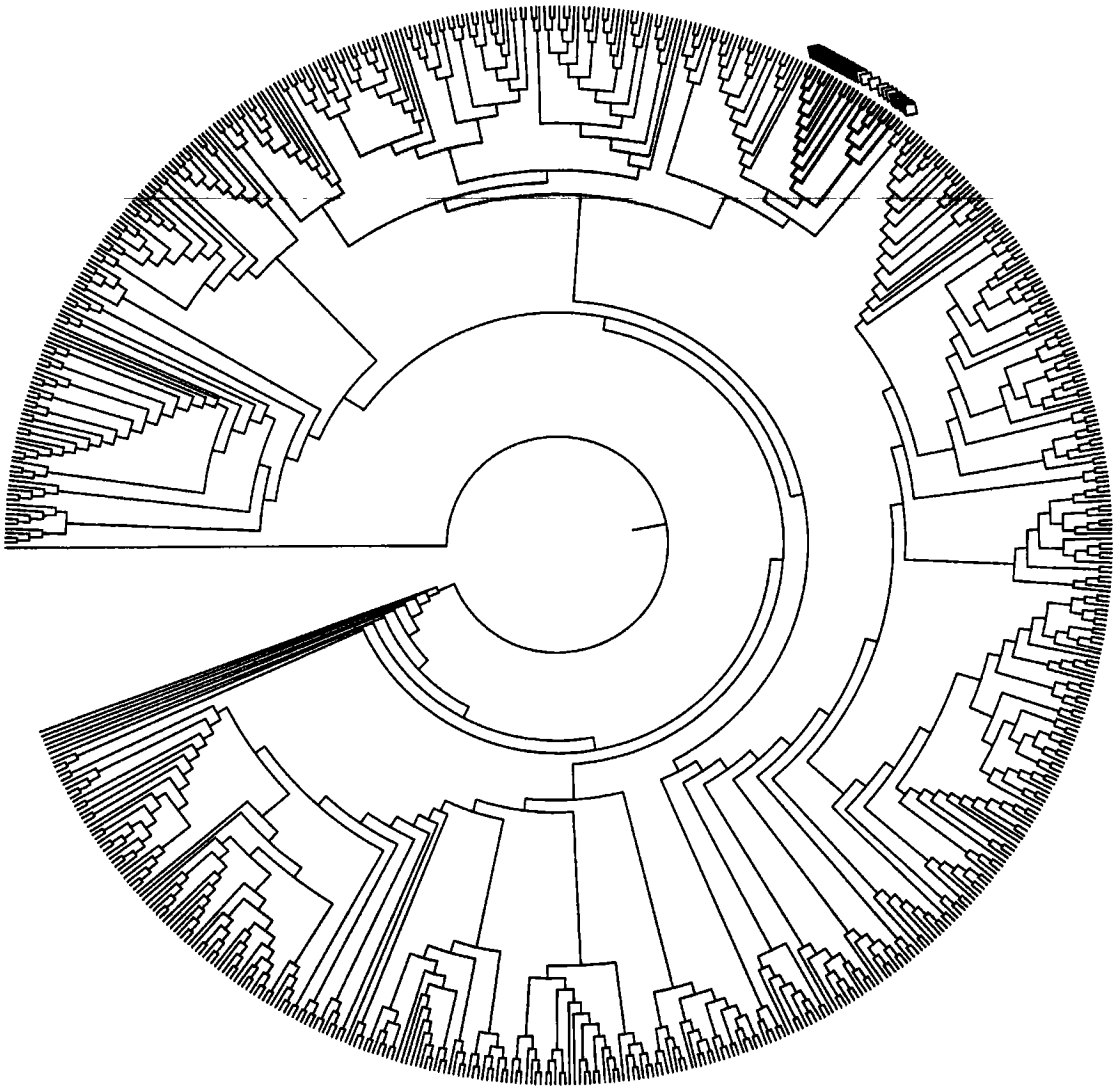
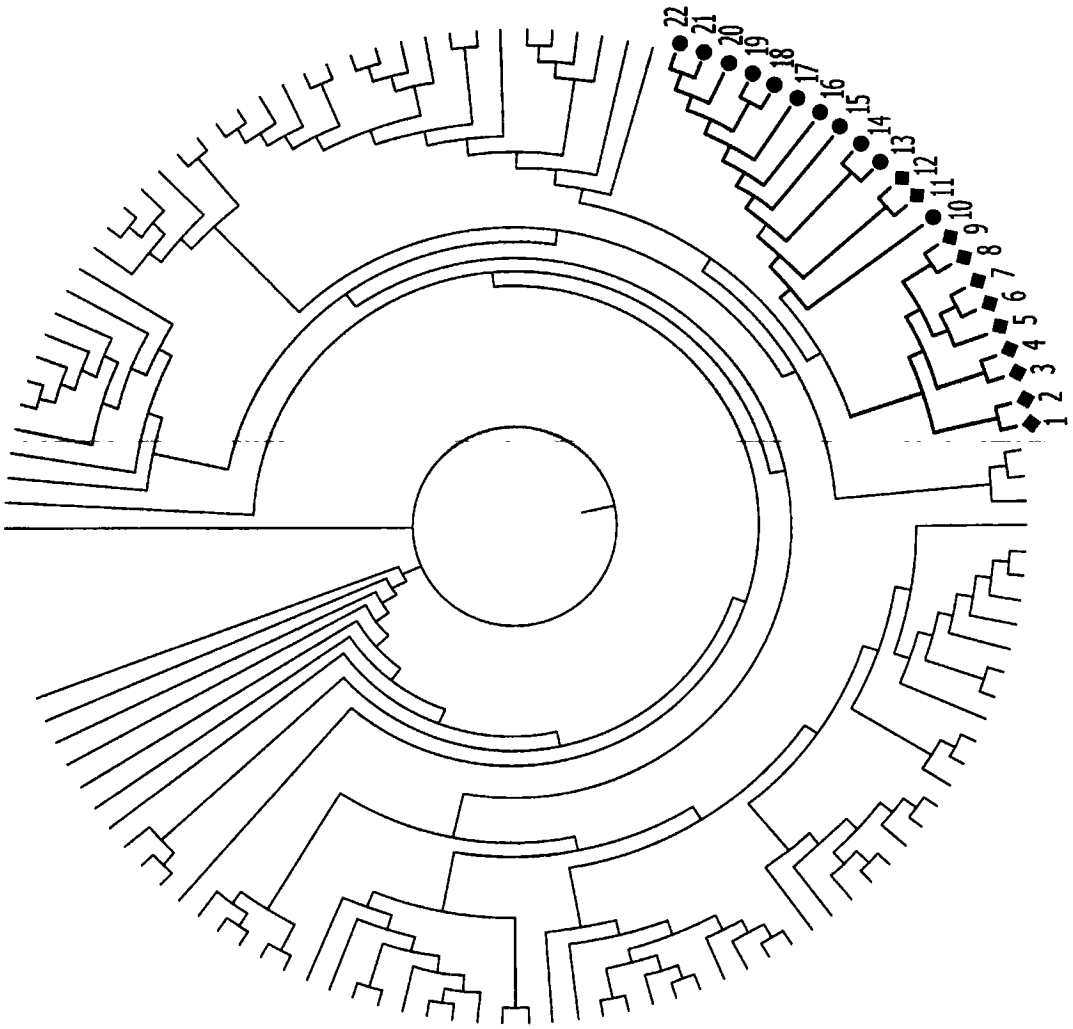


图 2



1	布氏乳杆菌
2	肠膜明串珠菌
3	乳酸乳球菌
4	乳酸乳球菌乳脂亚种MG1363
5	婴儿链球菌
6	猪链球菌
7	格登链球菌**
8	变形链球菌
9	前庭链球菌
10	溶酪巨型球菌
11	鹑鸡肠球菌
12	铅黄肠球菌
13	格氏李斯特菌
14	单核细胞增多性李斯特菌
15	肉葡萄球菌
16	腐生性葡萄球菌
17	表皮葡萄球菌W23144
18	沃氏葡萄球菌
19	金黄色葡萄球菌
20	人葡萄球菌
21	表皮葡萄球菌M23864-W1
22	头葡萄球菌
	**嗜热链球菌属于这个次分支

图 3

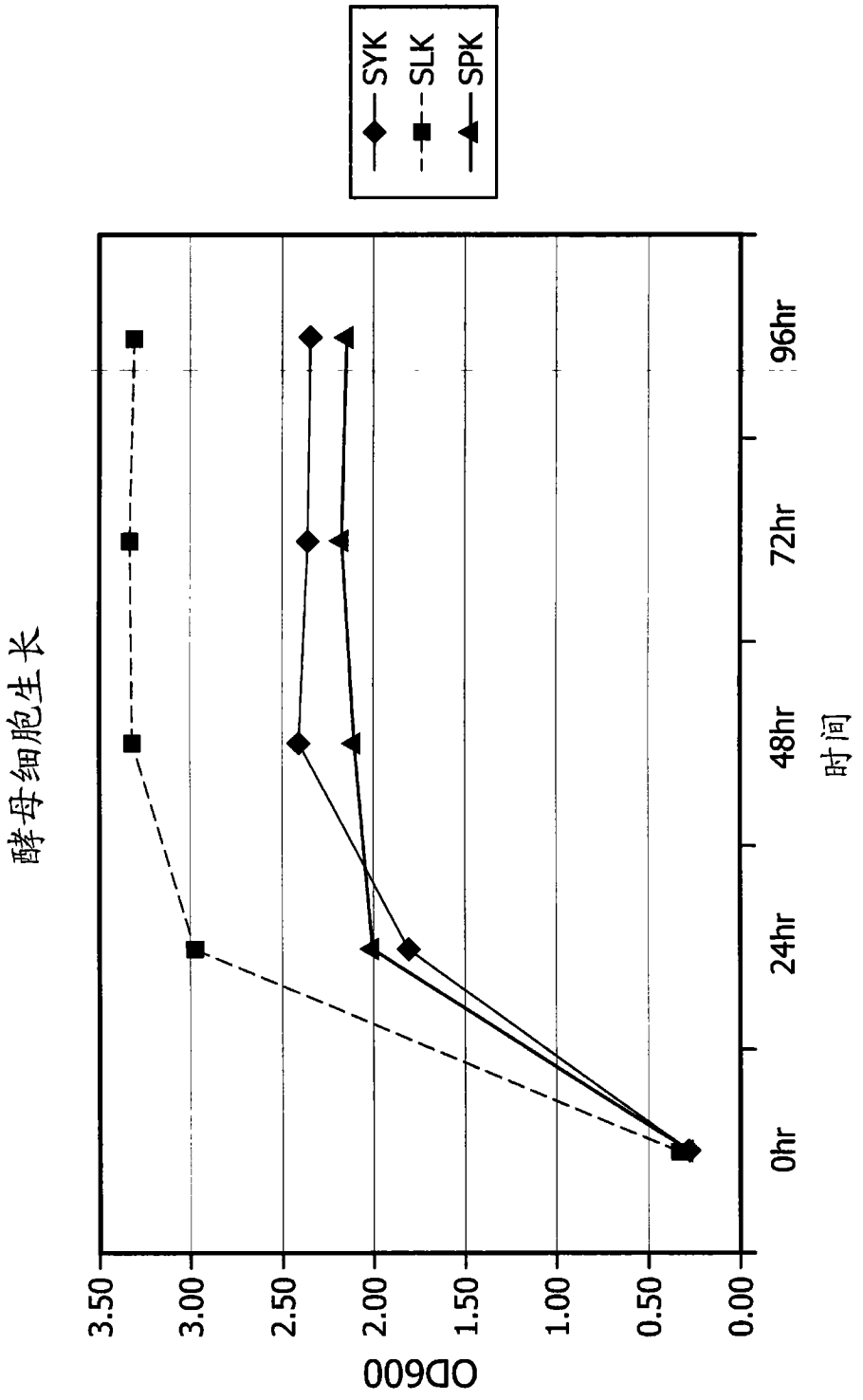

SYK
SLK
SPK
酵母细胞生长
OD600
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0hr
24hr
48hr
72hr
96hr
时间

图 4

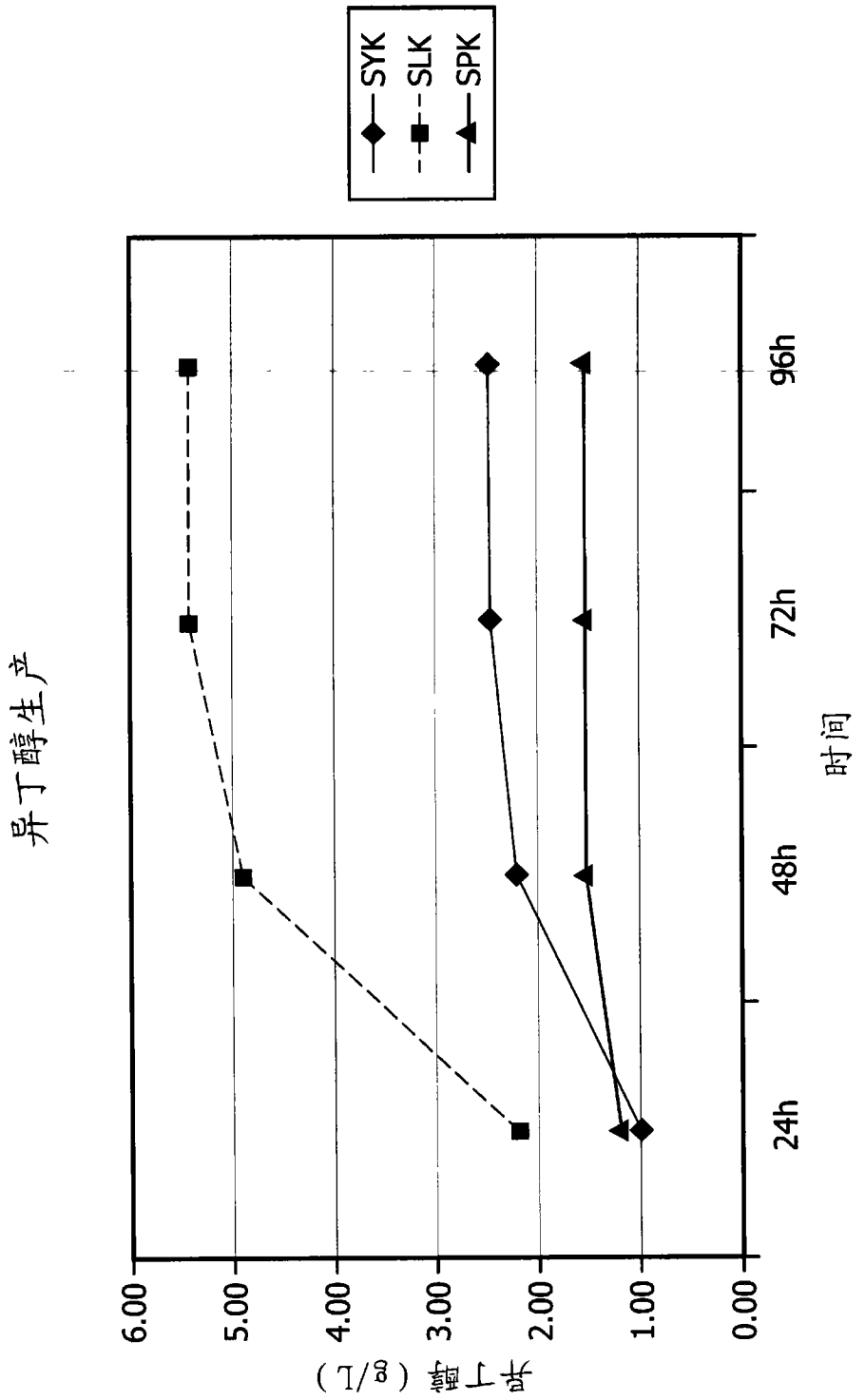

SYK
SLK
SPK
异丁醇生产
异丁醇 (g/L)
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5.00
4.00
3.00
2.00
1.00
0.00
24h
48h
72h
96h
时间

图 5