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(54)发明名称

消耗乙酸的菌株

(57)摘要

本发明描述了一种从包含葡萄糖和介于50 μ M与100mM之间的乙酸的组合物生产乙醇的方法,所述方法包括在能够厌氧地转化乙酸的重组酵母的存在下发酵所述组合物;将未离解的乙酸的量维持在至少50 μ M的值;以及回收乙醇。所述方法可用于基于淀粉和基于纤维素的含乙酸水解产物,并且有利地导致乙酸的更大消耗以及因此更高的乙醇产率。

1. 一种从包含葡萄糖和介于50 μ M与100mM之间的乙酸的组合物生产乙醇的方法,所述方法包括:

- 在能够厌氧地转化乙酸的重组酵母的存在下发酵所述组合物;
- 将未离解的乙酸的量维持在至少50 μ M的值;以及
- 回收乙醇。

2. 根据权利要求1所述的方法,其中将离解的乙酸的量维持在至少50 μ M的值的所述步骤包括:

-监测所述组合物中未离解的乙酸的量,以及如果所述未离解的乙酸的量降至低于50 μ M:

-向所述组合物中加入酸直到未离解的乙酸的量达到至少50 μ M的值,优选地通过加入酸。

3. 根据权利要求1所述的方法,其中将离解的乙酸的量维持在至少50 μ M的值的所述步骤包括:

-监测所述组合物中未离解的乙酸的量;以及如果未离解的乙酸的量接近50 μ M,但在所述量降至低于50 μ M之前:

-向所述组合物中加入酸直到未离解的乙酸的量达到高于50 μ M的值,优选地通过加入酸。

4. 根据权利要求1-3中任一项所述的方法,其中所述重组酵母包含:

- 编码具有乙酰化乙醛脱氢酶活性的酶(EC 1.2.1.10或EC 1.1.1.2)的核酸序列;
- 编码具有乙酰辅酶A合成酶活性的酶(E.C.6.2.1.1)的核酸序列;以及任选地
- 编码具有NAD依赖性醇脱氢酶活性的酶(EC 1.1.1.1)的核酸序列。

5. 根据权利要求1-4中任一项所述的方法,其中所述重组酵母包含编码具有甘油脱氢酶活性的酶的核酸序列。

6. 根据权利要求1-5中任一项所述的方法,其中所述重组酵母包含编码具有二羟基丙酮激酶活性的酶的核酸。

7. 根据权利要求1-6中任一项所述的方法,其中所述重组酵母包含编码甘油-3-磷酸脱氢酶的一种或多种内源核苷酸序列的缺失或破坏。

8. 根据权利要求1-7中任一项所述的方法,其中所述重组酵母包含编码甘油-3-磷酸磷酸水解酶诸如酿酒酵母(*S.cerevisiae*) GPP1或GPP2的一种或多种内源核苷酸序列的缺失或破坏。

9. 根据权利要求1-8中任一项所述的方法,其中所述重组酵母包含甘油转运体。

10. 根据权利要求1-9中任一项所述的方法,其中所述重组酵母包含编码醛脱氢酶的一种或多种内源核苷酸序列的缺失或破坏,或者所述酵母具有与其对应的野生型酵母相比降低的醛脱氢酶活性。

11. 根据权利要求10所述的方法,其中所述核苷酸序列编码醛脱氢酶ALD2、ALD3、ALD4、ALD5和/或ALD6,优选地ALD6。

12. 根据权利要求10或11所述的方法,其中所述酵母还包含:

-编码具有磷酸转酮酶(PKL)活性的酶(EC 4.1.2.9或EC 4.1.2.22)或具有根据SEQ ID NO:5、SEQ ID NO:6、SEQ ID NO:7或SEQ ID NO:8的氨基酸序列的酶或具有至少50%的序列

同一性的其功能同源物的一种或多种基因,以及

-编码具有磷酸转乙酰酶 (PTA) 活性的酶 (EC 2.3.1.8) 或具有根据SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11或SEQ ID NO:12的氨基酸序列的酶或具有至少50%的序列同一性的其功能同源物的一种或多种基因;和/或

-和/或编码具有乙酸激酶 (ACK) 活性的酶 (EC 2.7.2.12) 或具有根据SEQ ID NO:1或SEQ ID NO:2的氨基酸序列的酶或具有至少50%的序列同一性的其功能同源物的一种或多种基因。

13. 根据权利要求1-12中任一项所述的方法,其中所述组合物是木质纤维素生物质水解产物。

14. 根据权利要求1-13中任一项所述的方法,其中所述组合物是淀粉水解产物,诸如玉米淀粉水解产物。

15. 根据权利要求1-14中任一项所述的方法,其中所述酵母是酿酒酵母。

消耗乙酸的菌株

技术领域

[0001] 本发明涉及一种在能够将乙酸转化为乙醇的重组酵母的存在下从包含葡萄糖和乙酸的组合物生产乙醇的方法。

背景技术

[0002] 生物乙醇是由酿酒酵母 (*Saccharomyces cerevisiae*) 从一系列底物 (包括玉米淀粉、玉米纤维、和玉米秸秆) 以及还从非食品原料 (例如能源作物和农业残余物) 的其他木质纤维素水解产物产生的。这些生物质材料可包含乙酸, 所述乙酸作为材料本身的组分或由细菌污染形成。该乙酸形成了乙醇的潜在来源。

[0003] W02011/149353公开了一种酵母细胞, 所述酵母细胞包含编码具有乙醛脱氢酶活性的酶的外源基因, 所述基因赋予所述酵母细胞将乙酸转化为乙醇的能力。

[0004] 然而, 乙酸至乙醇的转化通常是不完全的。因此, 需要改进的乙酸转化。

[0005] 表1-序列表说明

[0006]

SEQ ID NO:	描述
1	大肠杆菌 (<i>E. coli</i>) 双功能 NAD ⁺ 依赖性乙酰化乙醛/醇脱氢酶 (adhE)
2	大肠杆菌乙醇胺利用蛋白 (eutE)
3	植物乳杆菌 (<i>L. plantarum</i>) 乙醛脱氢酶 (acdH)
4	英诺克李斯特菌 (<i>L. innocua</i>) 乙醛脱氢酶 (acdH)
5	金黄色葡萄球菌 (<i>S. aureus</i>) 乙醛/醇脱氢酶 (adhE)
6	大肠杆菌甘油脱氢酶 (gldA)
7	肺炎克雷伯氏菌 (<i>K. pneumoniae</i>) 甘油脱氢酶 (gldA)
8	产气肠杆菌 (<i>E. aerogenes</i>) 甘油脱氢酶 (gldA)

[0007]

9	阿氏耶尔森氏菌 (<i>Y. aldovae</i>) 甘油脱氢酶 (gldA)
10	酿酒酵母 (<i>S. cerevisiae</i>) 二羟基丙酮激酶 (DAK1)
11	肺炎克雷伯氏菌二羟基丙酮激酶 (dhaK)
12	解脂耶氏酵母 (<i>Y. lipolytica</i>) 二羟基丙酮激酶 (DAK1)
13	粟酒裂殖酵母 (<i>S. pombe</i>) 二羟基丙酮激酶 (DAK1)
14	斑马鱼 (<i>D. rerio</i>) 水通道蛋白 9 (T3)
15	鲁氏接合酵母 (<i>Z. rouxii</i>) ZYRO0E01210p (T5)
16	动物双歧杆菌 (<i>B. animalis</i>) 木酮糖-5P/果糖-6P 磷酸转酮酶
17	青春双歧杆菌 (<i>B. adolescentis</i>) 木酮糖-5P/果糖-6P 磷酸转酮酶
18	乳双歧杆菌 (<i>B. lactis</i>) 木酮糖-5P/果糖-6P 磷酸转酮酶
19	肠膜明串珠菌 (<i>L. mesenteroides</i>) 木酮糖-5P/果糖-6P 磷酸转酮酶
20	枯草芽孢杆菌 (<i>B. subtilis</i>) 磷酸转乙酰酶
21	植物乳杆菌磷酸转乙酰酶
22	青春双歧杆菌磷酸转乙酰酶
23	嗜热毁丝霉 (<i>M. thermophila</i>) 磷酸转乙酰酶
24	青春双歧杆菌乙酸激酶
25	构巢曲霉 (<i>A. nidulans</i>) 乙酸激酶
26	酿酒酵母醛脱氢酶 ALD2
27	酿酒酵母醛脱氢酶 ALD3
28	酿酒酵母醛脱氢酶 ALD4
29	酿酒酵母醛脱氢酶 ALD5
30	酿酒酵母醛脱氢酶 ALD6

[0008] 发明概述

[0009] 本发明描述了一种从包含葡萄糖和介于50 μ M与100mM之间的乙酸的组合物生产乙醇的方法,所述方法包括在能够厌氧地转化乙酸的重组酵母的存在下发酵所述组合物;将未离解的乙酸的量维持在至少50 μ M的值;以及回收乙醇。所述方法可用于基于淀粉和基于纤维素的含乙酸水解产物,并且有利地导致乙酸的更大消耗以及因此更高的乙醇产率。将离解的乙酸的量维持在至少50 μ M的值的步骤可包括:监测组合物中未离解的乙酸的量;以及如果未离解的乙酸的量降至低于50 μ M;向所述组合物中加入酸直到未离解的乙酸的量达到至少50 μ M的值,优选地通过加入酸。或者,将未离解的乙酸的量维持在至少50 μ M的值的步骤可包括监测组合物中未离解的乙酸的量;以及如果未离解的乙酸的量接近50 μ M,但在所述量降至低于50 μ M之前:向所述组合物中加入酸直到未离解的乙酸的量达到高于50 μ M的值,优选地通过加入酸。重组酵母可包含编码具有乙酰化乙醛脱氢酶活性的酶 (EC

1.2.1.10或EC 1.1.1.2)的核酸序列;编码具有乙酰辅酶A合成酶活性的酶(E.C.6.2.1.1)的核酸序列,以及任选地编码具有NAD依赖性醇脱氢酶活性的酶(EC 1.1.1.1)的核酸序列。重组酵母还可包含编码具有甘油脱氢酶活性的酶的核酸序列。重组酵母可包含编码醛脱氢酶的一种或多种内源核苷酸序列的缺失或破坏,或者所述重组酵母可具有与其对应野生型酵母相比降低的醛脱氢酶活性。此类细胞还可包含编码具有磷酸转酮酶(PKL)活性的酶(EC 4.1.2.9或EC 4.1.2.22)的一种或多种基因、编码具有磷酸转乙酰酶(PTA)活性的酶(EC 2.3.1.8)的一种或多种基因,和/或编码具有乙酸激酶(ACK)活性的酶(EC 2.7.2.12)的一种或多种基因。重组酵母还可包含编码具有二羟基丙酮激酶活性的酶的核酸。重组酵母还可包含编码甘油-3-磷酸脱氢酶的一种或多种内源核苷酸序列的缺失或破坏。重组酵母还可以包含编码甘油-3-磷酸磷酸水解酶的一种或多种内源核苷酸序列(诸如酿酒酵母GPP1或GPP2)的缺失或破坏。重组酵母还可包含甘油转运体。所述组合物可以是木质纤维素生物质水解产物或淀粉水解产物,诸如玉米淀粉水解产物。酵母可以是酿酒酵母。

[0010] 发明详述

[0011] 酵母中外源乙酰化乙醛脱氢酶的表达允许酵母将木质纤维素水解产物和玉米淀粉水解产物中可存在的乙酸转化为乙醇。已提议将乙酸至乙醇的NADH依赖性还原作为在酿酒酵母的厌氧葡萄糖生长培养中作为氧化还原池(redox sink)的甘油形成的替代方案,从而为消除在工业乙醇生产期间的甘油产生(作为副产物)以及因此更高的乙醇产量提供化学计量基础。然而,本发明人已惊奇地发现,当使用此类酵母时,在发酵培养基中往往存在保持不转化的残留量的乙酸。乙酸的该残留量可高达几毫摩尔。本发明人惊奇地发现,通过加入少量的酸,酵母恢复了对乙酸的消耗。意识到加入酸会迫使乙酸变成其质子化(即未离解)形式,本发明人推断酵母消耗乙酸可能需要存在最低浓度的未离解乙酸。本发明人确实发现,酵母需要至少50 μ M的最低浓度的未离解乙酸—如果该量降至低于50 μ M,则乙酸的消耗逐渐减少并最终完全停止,即使发酵培养基中存在可观量的离解的乙酸根也如此。如果是这种情况,将未离解的乙酸的量增加到至少50 μ M的值,则乙酸消耗将接近完全。本发明人认识到,酵母仅消耗未离解的乙酸,而不消耗乙酸的离解形式,并且进一步地,酵母仅可消耗浓度达约50 μ M的此类未离解的乙酸。明显地,当未离解的乙酸的量降至低于50 μ M的浓度时,酵母完全停止消耗乙酸,即使发酵培养基中存在可观的乙酸根储量也如此。

[0012] 因此,本发明涉及一种从包含糖和介于50 μ M与100mM之间的乙酸的组合物生产乙醇的方法,所述方法包括:

[0013] -在能够厌氧地转化乙酸的重组酵母的存在下发酵所述组合物;

[0014] -将离解的乙酸的量维持在至少50 μ M的值;以及

[0015] -回收乙醇。

[0016] 可以将未离解的乙酸的量维持在至少60 μ M、至少70 μ M、至少80 μ M、至少90 μ M、至少100 μ M、或至少120 μ M、至少150 μ M,或至少200 μ M的值。

[0017] 未离解的乙酸的上限量不是很重要,但是可以是100mM或更低,优选地80mM或更低、60mM或更低、40mM或更低、30mM或更低、20mM或更低、10mM或更低、5mM或更低、2mM或更低、1mM或更低。如果未离解的乙酸的量太高,例如100mM,则结果可能是减少的生长、不完全发酵和/或降低的乙醇产率,这可能是由于乙酸毒性。由于预料到在乙酸转化为乙醇的发酵过程期间pH会增大,因此未离解的乙酸的量也将减少。因此,只要组合物中乙酸的起始浓度

不高于100mM,未离解的乙酸的量就不会增加到高于100mM,并且不需要采取主动步骤来防止未离解的乙酸的增加到高于100mM的水平。假设地,组合物中几乎所有的乙酸都是未离解的形式,但是这将需要非常低的pH值,而非常低的pH值不利于酵母。因此,在一个实施方式中,将未离解的乙酸的量维持在100mM或更低,优选地80mM或更低、60mM或更低、40mM或更低、30mM或更低、20mM或更低、10mM或更低、5mM或更低、2mM或更低、1mM或更低的值,例如通过加入碱,诸如NaOH或KOH。

[0018] 在本发明的方法中,预期作为乙酸消耗的结果,在发酵期间pH增大。因此,在一个实施方式中,本发明的方法导致组合物的pH增大。

[0019] 在一个实施方式中,乙酸的至少一部分转化为乙醇。

[0020] 在整个说明书和所附权利要求书中,词语“包括”和“包含”将被包含性地解释。也就是说,在上下文允许的情况下,这些词语旨在传达可包括未具体叙述的其他要素或整数。不使用数量词修饰在本文中用于指代一个(种)或多于一个(种)(即,一个(种)或至少一个(种))的语法对象。举例来说,“氨基酸”可以表示一种氨基酸或多于一种氨基酸。

[0021] 在本发明的上下文中,“将离解的乙酸的量维持在至少50 μ M的值”并不意味着未离解的乙酸的量必须一直不能降至低于50 μ M。确实,在发酵过程期间,可发生的是未离解的乙酸的量偶尔降至低于50 μ M。当这种情况发生时,所述方法需要将未离解的乙酸的量恢复至至少50 μ M的值。在这种情况下,在浓度降至低于50 μ M之后采取步骤以实现至少50 μ M的量的未离解的乙酸。在离解的乙酸的量已降至低于50 μ M的值的时刻与将离解的乙酸的量恢复到至少50 μ M的值的时刻之间的时间并不重要。这可以立即进行,或在1分钟之后、5分钟之后,甚至30分钟或1小时之后进行。

[0022] 替代地,还可以进行“维持”以使得未离解的乙酸的量不降至低于50 μ M。

[0023] 在这两种情况下,在发酵过程期间监测未离解的乙酸的量是有利的。

[0024] 在本发明的上下文中,术语“未离解的乙酸”应理解为与“质子化的乙酸”相同。

[0025] 技术人员知道如何将未离解的乙酸的量维持在介于50 μ M与100mM之间。他/她可以监测未离解的乙酸的量。因此,在一个实施方式中,该方法包括监测未离解的乙酸的量的步骤。

[0026] 技术人员理解的是,未离解的乙酸的量尤其取决于组合物中乙酸(质子化和离解的)的总量以及pH。因此,在一个实施方式中,通过调节pH将离解的乙酸的量维持在至少50 μ M的值。由于乙酸的消耗通常导致pH增大,并且由于未离解的乙酸量尤其取决于pH,因此在较高的pH下未离解的乙酸的量减少,当未离解的乙酸的量降至低于50mM时,可降低组合物的pH。这可以通过加入酸来完成。因此,在一个实施方式中,所述方法包括加入酸。任何类型的酸都可以满足要求,例如有机酸,诸如柠檬酸;或无机酸,诸如盐酸、硫酸或硝酸。优选的是强酸,因为弱酸可能抑制酵母。磷酸非常合适,并且可具有向发酵培养基补充磷酸根的附加效应。在发酵期间酸可以加入一次,或(取决于乙酸的量)加入两次、三次等。

[0027] 可以加入酸直到未离解的乙酸的量达到50 μ M或更高的值,诸如介于50 μ M与60 μ M之间的值,或介于50 μ M与70 μ M之间、或介于50 μ M与100 μ M之间、或介于50 μ M与150 μ M之间,或介于50 μ M与200 μ M之间的值。

[0028] 所述方法还可以包括监测pH的步骤。将组合物的pH优选地保持在介于3与6之间,优选地介于4与5之间。pH的上限优选地使得未离解的乙酸的量是至少50 μ M,并且这取决于

组合物中乙酸的总量。本领域的技术人员可以容易地监测pH和未离解的乙酸的量两者(见下文),并调节pH以使得未离解的乙酸的量维持在(或恢复到)至少50 μ M的值。

[0029] 未离解的乙酸的量可以通过HPLC分析。HPLC通常测量所有的乙酸和乙酸盐(即乙酸的未离解(即质子化)形式和离解形式两者),因为流动相通常被酸化。为了测量组合物中离解的乙酸的量,合适的方法是测量原样组合物的乙酸的(总)量,测量组合物的pH,并使用乙酸的pKa计算离解的乙酸的量。

[0030] 因此,在一个实施方式中,所述方法包括:

[0031] -监测组合物中未离解的乙酸的量,以及如果未离解的乙酸的量降至低于50 μ M

[0032] -向所述组合物中加入酸直到未离解的乙酸的量达到至少50 μ M的值,优选地通过加入酸。

[0033] 在另一个实施方式中,所述方法包括:

[0034] -监测组合物中未离解的乙酸的量;以及如果未离解的乙酸的量接近50 μ M,但在所述量降至低于50 μ M之前:

[0035] -向所述组合物中加入酸直到未离解的乙酸的量达到高于50 μ M的值,优选地通过加入酸。

[0036] 为避免任何疑惑,本发明还包括一种从包含糖和介于50 μ M与100mM之间的乙酸的组合物生产乙醇的方法,所述方法包括:

[0037] -在能够厌氧地转化乙酸的重组酵母的存在下发酵所述组合物;

[0038] -监测组合物中未离解的乙酸的量;以及如果未离解的乙酸的量接近50 μ M,但在所述量降至低于50 μ M之前:

[0039] -向所述组合物中加入酸直到未离解的乙酸量达到高于50 μ M(诸如55 μ M或更高、60 μ M或更高、70 μ M或更高、80 μ M或更高、90 μ M或更高、100 μ M或更高)的值,优选地通过加入酸;以及

[0040] -回收乙醇产物。

[0041] 乙酰化乙醛脱氢酶(EC1.2.1.10或EC1.1.1.2)催化乙酰辅酶A转化为乙醛。该转化可通过平衡反应式(I)表示:

[0042] (I) 乙酰辅酶A+NADH+H⁺ $\rightleftharpoons$ 乙醛+NAD⁺+辅酶A

[0043] 应当理解,在本发明的方法中使用的重组酵母天然地包含编码乙酰辅酶A合成酶的至少一种内源基因和编码醇脱氢酶的至少一种内源基因。因此,在本发明的上下文中,用编码乙酰化乙醛脱氢酶的基因转化并具有编码乙酰辅酶A合成酶和醇脱氢酶的内源基因的该重组酵母能够完成乙酸至乙醇的转化。

[0044] 在一个实施方式中,重组酵母包含:

[0045] -编码具有乙酰化乙醛脱氢酶活性的酶(EC 1.2.1.10或EC 1.1.1.2)的核酸序列;

[0046] -编码具有乙酰辅酶A合成酶活性的酶(E.C.6.2.1.1)的核酸序列;以及任选地

[0047] -编码具有NAD依赖性醇脱氢酶活性的酶(EC 1.1.1.1)的核酸序列。

[0048] 所述方法中使用的重组酵母能够厌氧地转化乙酸。在一个实施方式中,重组酵母能够将乙酸转化为至少乙醇。在另一个实施方式中,重组酵母能够将葡萄糖和乙酸中的每一者转化为至少乙醇。在本发明的上下文中,术语“能够转化乙酸”和“能够将葡萄糖和乙酸中的每一者转化”应理解为是指将乙酸和/或葡萄糖中的至少部分转化为至少乙醇。

[0049] 编码具有乙酰化乙醛脱氢酶活性的酶的核酸优选是NAD⁺依赖性的,并且可以具有根据SEQ ID NO:1、SEQ ID NO:2、SEQ ID NO:3、SEQ ID NO:4或SEQ ID NO:5的氨基酸序列或其具有至少50%,优选地至少60%、70%、75%、80%、85%、90%或95%的序列同一性的功能同源物,或者通过一个或多个氨基酸替换、缺失或插入从SEQ ID NO:1、SEQ ID NO:2、SEQ ID NO:3、SEQ ID NO:4或SEQ ID NO:5的氨基酸序列得到的功能同源物。乙酰化乙醛可包含NAD⁺依赖性乙酰化乙醛脱氢酶(EC 1.2.1.10或EC 1.1.1.2)活性和NAD⁺依赖性醇脱氢酶(EC 1.1.1.1)活性两者。编码NAD⁺依赖性乙酰化乙醛脱氢酶的核酸序列原则上可以来源于包含编码所述脱氢酶的核酸序列的任何生物。能够催化乙酰辅酶A至乙醛的NADH依赖性还原的已知乙酰化乙醛脱氢酶通常可以分为三种类型的NAD⁺依赖性乙酰化乙醛脱氢酶功能同源物:

[0050] 1) 催化乙酰辅酶A可逆转化为乙醛,以及随后乙醛可逆转化为乙醇的双功能蛋白。这类蛋白质的示例是大肠杆菌中的AdhE蛋白质(GenBank编号:NP_415757)。AdhE表现为是基因融合的进化产物。AdhE蛋白的NH₂末端区域与醛:NAD⁺氧化还原酶高度同源,而COOH末端区域与Fe²⁺依赖性乙醇:NAD⁺氧化还原酶家族同源(Memorial-Hernandez等人,(2000) J.Biol.Chem.275:33869-33875)。大肠杆菌AdhE经受金属催化氧化,因此对氧敏感(Tamarit等人,(1998) J.Biol.Chem.273:3027-32)。

[0051] 2) 在严格或兼性厌氧微生物中催化将乙酰辅酶A可逆转化为乙醛但不具有醇脱氢酶活性的蛋白质。在克氏梭菌(*Clostridium kluyveri*)中已经报道了这种类型的蛋白质的示例(Smith等人,(1980) Arch.Biochem.Biophys.203:663-675)。在克氏梭菌DSM 555(GenBank编号:EDK33116)的基因组中已经注释了乙酰化乙醛脱氢酶。在植物乳杆菌的基因组中标识了同源蛋白质AcdH(GenBank编号:NP_784141)。这种类型的蛋白质的另一个示例是在拜氏梭菌(*Clostridium beijerinckii*) NRRL B593中的所述基因产物(Toth等人,(1999) Appl.Environ.Microbiol.65:4973-4980,GenBank编号:AAD31841)。

[0052] 3) 作为参与4-羟基-2-酮戊酸酯分解代谢的双功能醛缩酶-脱氢酶复合物的部分的蛋白质。此类双功能酶催化儿茶酚的间位切割途径的最后两个步骤,儿茶酚是许多细菌物种中酚类、甲苯甲酸酯、萘、联苯和其他芳香族化合物降解的中间体(Powlowski和Shingler(1994) Biodegradation 5,219-236)。首先通过4-羟基-2-酮戊酸酯醛缩酶将4-羟基-2-酮戊酸酯转化为丙酮酸酯和乙醛,随后通过乙酰化乙醛脱氢酶将乙醛转化为乙酰辅酶A。这种类型的乙酰化乙醛脱氢酶的示例是假单胞菌属(*Pseudomonas* sp.) CF600中的DmpF蛋白质(GenBank编号:CAA43226)(Shingler等人,(1992) J.Bacteriol.174:711-24)。大肠杆菌MphF蛋白质(Ferrandez等人,(1997) J.Bacteriol.179:2573-2581,GenBank编号:NP_414885)与假单胞菌属CF600中的DmpF蛋白质同源。

[0053] 合适的核酸序列可以尤其存在于选自以下组的生物中:埃希氏菌属(*Escherichia*),尤其是大肠杆菌;分枝杆菌属(*Mycobacterium*),尤其是海分枝杆菌(*Mycobacterium marinum*)、溃疡分枝杆菌(*Mycobacterium ulcerans*)、结核分枝杆菌(*Mycobacterium tuberculosis*);氧化碳嗜热菌属(*Carboxydotherrmus*),尤其是生氢氧化碳嗜热菌(*Carboxydotherrmus hydrogenoformans*);内变形虫属(*Entamoeba*),尤其是痢疾变形虫(*Entamoeba histolytica*);志贺氏菌属(*Shigella*),尤其是宋内志贺菌(*Shigella sonnei*);伯克霍尔德菌属(*Burkholderia*),尤其是类鼻疽伯克霍尔德菌(*Burkholderia*

pseudo mallei);克雷伯氏菌属(*Klebsiella*),尤其是肺炎克雷伯氏菌(*Klebsiella pneumoniae*);固氮菌属(*Azotobacter*),尤其是棕色固氮菌(*Azotobacter vinelandii*);固氮弧菌属(*Azoarcus* sp);贪铜菌属(*Cupriavidus*),尤其是台湾贪铜菌(*Cupriavidus taiwanensis*);假单胞菌属(*Pseudomonas*),尤其是假单胞菌属(*Pseudomonas* sp.)CF600; *Pelomaculum*,尤其是*Pelotomaculum thermopropionicum*。优选地,编码NAD⁺依赖性乙酰化乙醛脱氢酶的核酸序列源自埃希氏菌属,更优选地源自大肠杆菌。

[0054] 特别合适的是来自大肠杆菌的mhpF基因,或其功能同源物。该基因描述于Fernindez等人,(1997) *J. Bacteriol.* 179:2573-2581中。已经利用酿酒酵母获得了良好的结果,其中已整合了来自大肠杆菌的mhpF基因。在另一个有利的实施方式中,编码(乙酰化)乙醛脱氢酶的核酸序列来自假单胞菌属,尤其是(例如来自假单胞菌属CF600的)dmpF。

[0055] 编码乙酰化乙醛脱氢酶的核酸序列可以是野生型核酸序列。此外,乙酰化乙醛脱氢酶(或编码此类活性的核酸序列)可以例如选自以下项的组:大肠杆菌adhE、痢疾变形虫adh2、金黄色葡萄球菌(*Staphylococcus aureus*) adhE、梨囊鞭菌属(*Piromyces* sp.) E2 adhE、克氏梭菌EDK33116、植物乳杆菌acdH、大肠杆菌eutE、无害李斯特菌(*Listeria innocua*) acdH和恶臭假单胞菌(*Pseudomonas putida*) YP 001268189。对于这些酶中的一些酶的序列,编码这些酶的核酸序列以及将所述核酸序列掺入宿主细胞中的方法,请参考W02009/013159,特别是实施例3,表1(第26页)和其中提及的序列ID编号,其中表1和所述表中提及的序列ID编号表示的序列的公开通过引用方式并入本文。

[0056] 乙酰辅酶A合成酶(也称为乙酸辅酶A连接酶和乙酰活化酶)是一种存在于原核生物和真核生物两者中的普遍存在的酶,其催化从乙酸、辅酶A (CoA) 和ATP形成乙酰辅酶A,如下所示:

[0057] (II) $\text{ATP} + \text{乙酸} + \text{CoA} = \text{AMP} + \text{二磷酸} + \text{乙酰辅酶A}$

[0058] 该酶的活性对于维持所需水平的乙酰辅酶A至关重要,乙酰辅酶A是许多重要的生物合成和分解代谢过程中的关键中间体。这在真核物种中尤其重要,因为这是在生物中将乙酸活化为乙酰辅酶A的唯一途径(一些原核物种也可以通过乙酸激酶/磷酸转乙酰酶或通过形成ADP的乙酰辅酶A合成酶来活化乙酸)。真核生物通常具有两种乙酰辅酶A合成酶同种型,参与生物合成过程的胞质形式以及主要参与能量生成的线粒体形式。

[0059] 已经确定了该酶的真核(例如来自酵母)和细菌(例如来自沙门氏菌属(*Salmonella*))形式的晶体结构。酵母酶是三聚体,而细菌酶是单体。然而,由于三聚体界面中涉及的残基在其他序列中的保守性很差,因此酵母蛋白质的三聚体状态可能是该生物体所独有的。尽管两种酶的寡聚状态不同,但单体的结构几乎相同。包含两个平行 β 折叠的大N末端结构域(约500个残基)之后是包含带有螺旋的三链 β 折叠的小(约110个残基)C末端结构域。活性位点出现在结构域界面处,其内容确定了C末端结构域的定向。

[0060] 重组酵母可包含过表达的内源ACS。重组细胞可以包含异源ACS。表2列出了合适的异源ACS的示例。

[0061] 表2:BLAST查询—来自酿酒酵母的ACS2

	描述	同一性(%)	登录号
	乙酸-辅酶 A 连接酶 ACS2 [酿酒酵母 S288c]	100	NP_013254.1
	乙酰辅酶 A 合成酶[酿酒酵母 YJM789]	99	EDN59693.1
	乙酸-辅酶 A 连接酶[乳酸克鲁维酵母 (<i>Kluyveromyces lactis</i>) NRRL Y-1140]	85	XP_453827.1
[0062]	乙酸-辅酶 A 连接酶[光滑念珠菌 (<i>Candida glabrata</i>) CBS 138]	83	XP_445089.1
	乙酸-辅酶 A 连接酶[树干舍弗氏菌 (<i>Scheffersomyces stipitis</i>) CBS 6054]	68	XP_001385819.1
	乙酰辅酶 A 合成酶 <i>FacA</i> [烟曲霉菌 (<i>Aspergillus fumigatus</i>) A1163]	63	EDP50475.1
	乙酸-辅酶 A 连接酶 <i>facA</i> -产黄青霉菌 (<i>Penicillium chrysogenum</i>) [产黄青霉菌威斯康星 (<i>Penicillium chrysogenum Wisconsin</i>) 54-1255]	62	XP_002564696.1

[0063] 在一个实施方式中,重组酵母可包含编码具有NAD⁺连接的甘油脱氢酶的酶的核苷酸。如本文所用,甘油脱氢酶至少催化以下反应:

[0064] (III) 甘油+NAD⁺↔甘油酮+NADH+H⁺

[0065] 因此,该酶的两种底物是甘油和NAD⁺,而其三种产物是甘油酮、NADH和H⁺。甘油酮和二羟基丙酮在本文中是同义词。

[0066] 该酶属于氧化还原酶家族,特别是以NAD⁺或NADP⁺为受体的作用于供体的CH-OH基团的那些。该酶类别的系统名称是甘油:NAD⁺2-氧化还原酶。常用的其他名称包括丙三醇脱氢酶和NAD⁺连接的甘油脱氢酶。该酶参与甘油脂代谢。结构研究已经表明,该酶是锌依赖性的,其活性位点位于蛋白质的两个结构域之间。

[0067] 在一个实施方式中,具有甘油脱氢酶活性的酶优选地是NAD⁺连接的甘油脱氢酶(EC 1.1.1.6)。此类酶可以来自细菌来源或例如来自真菌来源。一个示例是来自大肠杆菌的gldA。

[0068] 或者,具有甘油脱氢酶活性的酶是NADP⁺连接的甘油脱氢酶(EC1.1.1.72)。

[0069] 当重组酵母用于通常在厌氧条件下进行的乙醇生产时,NAD⁺连接的甘油脱氢酶是优选的。

[0070] 在一个实施方式中,重组酵母包含编码由氨基酸序列SEQ ID NO:6、SEQ ID NO:7、SEQ ID NO:8或SEQ ID NO:9表示的异源甘油脱氢酶或,具有至少50%,优选地至少60%、70%、75%、80%、85%、90%或95%的序列同一性的其功能同源物,或者通过一个或多个氨基酸替换、缺失或插入从SEQ ID NO:6、SEQ ID NO:7、SEQ ID NO:8或SEQ ID NO:9的氨基酸序列得到的功能同源物的基因。

[0071] 重组酵母包含编码具有二羟基丙酮激酶活性的酶的核酸。二羟基丙酮激酶催化以下反应中的至少一个:

[0072] (IV) EC 2.7.1.28:ATP+D-甘油醛 $\rightleftharpoons$ ADP+D-甘油醛3-磷酸

[0073] 或者

[0074] (V) EC 2.7.1.29:ATP+甘油酮 $\rightleftharpoons$ ADP+磷酸甘油酮

[0075] 该家族由使用ATP (EC 2.7.1.29或EC 2.7.1.28) 而不是如在大肠杆菌中的磷蛋白质作为磷酸供体的二羟基丙酮激酶 (也称为甘油酮激酶) 的单链形式的示例组成。该形式具有与大肠杆菌酶的K亚基和L亚基同源的可分离结构域, 并且存在于酵母和其他真核生物中以及一些细菌 (包括弗氏柠檬酸杆菌 (*Citrobacter freundii*)) 中。来自番茄的成员已显示为使二羟基丙酮、3,4-二羟基-2-丁酮以及一些其他醛糖和酮糖磷酸化。来自哺乳动物的成员已显示为催化二羟基丙酮的磷酸化和核糖核苷二磷酸-X化合物的分割, 其中FAD是最好的底物。在酵母中, 存在两种二羟基丙酮激酶同工酶 (Dak1和Dak2)。在一个实施方式中, 重组酵母包含过表达的内源DAK。

[0076] 具有二羟基丙酮激酶活性的酶可以由内源基因 (例如DAK1) 编码, 所述内源基因优选地置于组成型启动子的控制下。重组细胞可包含增大细胞中二羟基丙酮激酶的比活性的遗传修饰。

[0077] 在一个实施方式中, 重组酵母包含编码由根据SEQ ID NO:10、SEQ ID NO:11、SEQ ID NO:12或SEQ ID NO:13的氨基酸序列表示的二羟基丙酮激酶或具有至少50%, 优选地至少60%、70%、75%、80%、85%、90%或95%的序列同一性的其功能同源物, 或通过一个或多个氨基酸替换、缺失或插入从SEQ ID NO:10、SEQ ID NO:11、SEQ ID NO:12或SEQ ID NO:13的氨基酸序列得到的功能同源物的一种或多种核酸序列, 所述基因优选地置于组成型启动子的控制下。

[0078] 在一个实施方式中, 重组酵母包含编码甘油-3-磷酸脱氢酶的一种或多种内源核苷酸序列的缺失或破坏。此类缺失或破坏可导致酶促活性的降低或去除。如本文所用, 甘油-3-磷酸脱氢酶至少催化以下反应:

[0079] (VI) 磷酸二羟丙酮+NADH $\rightarrow$ 磷酸甘油+NAD⁺

[0080] 可以使甘油-3-磷酸脱氢酶完全缺失, 或者缺失编码对酶活性必不可少的酶部分的至少一部分。特别地, 用酿酒酵母细胞已经实现了良好的结果, 其中GPD1基因和GPD2基因的开放阅读框已经失活。本领域技术人员可以通过合成地合成或以其他方式构建这样的DNA片段来实现结构基因 (靶基因) 的失活, 该DNA片段由侧翼为与宿主细胞基因组的待缺失区域侧翼的序列相同的DNA序列的选择性标记基因组成。特别地, 通过对标记基因kanMX和hphMX4的整合, 对酿酒酵母中GPD1和GPD2基因的失活已经获得了良好的结果。随后将该DNA片段转化到宿主细胞中。检查表达显性标记基因的转化细胞中对被设计为要缺失的区域的正确替换, 例如通过诊断性聚合酶链式反应或Southern杂交。被缺失或破坏的甘油-3-磷酸脱氢酶优选属于EC 1.1.5.3, 诸如GUT2, 或属于EC 1.1.1.8, 诸如GPD1和/或GPD2。在一个实施方式中, 细胞不含编码NADH依赖性甘油-3-磷酸脱氢酶的基因。GPD1和GPD2基因均可以缺失或破坏, 但是优选的是GPD2, 而不是GPD1被缺失或破坏。WO2011/010923描述了缺失或破坏甘油-3-磷酸脱氢酶的方法。

[0081] 在一个实施方式中, 重组酵母包含编码甘油-3-磷酸磷酸水解酶的一个或多个内源核苷酸序列 (诸如酿酒酵母GPP1或GPP2) 的缺失或破坏。此类缺失或破坏可导致酶促活性的降低或去除。

[0082] 在一个实施方式中,重组细胞包含编码甘油转运体的一种或多种基因。在培养基中的可外部获得的(例如从玉米醪液中的回流物(backset))或在内部细胞合成后分泌的甘油可以运输到细胞中,并通过甘油脱氢酶和二羟基丙酮激酶的同时过表达转化为乙醇。在一个实施方式中,重组细胞包含编码由SEQ ID NO:14或SEQ ID NO:15表示的异源甘油转运体或具有至少60%,优选地至少70%、75%、80%、85%、90%或95%的序列同一性的其功能同源物,或者通过一个或多个氨基酸替换、缺失或插入从SEQ ID NO:14或SEQ ID NO:15的氨基酸序列得到的功能同源物的基因。

[0083] 在一个实施方式中,所述组合物是生物质水解产物。此类生物质水解产物可以是木质纤维素生物质水解产物。本文的木质纤维素包括半纤维素和生物质的半纤维素部分。木质纤维素还包括生物质的木质纤维素级分。合适的木质纤维素材料可在以下列表中找到:果园底料(orchard primings)、树丛(chaparral)、磨坊废弃物(mill waste)、城市木材废弃物、市政废弃物、伐木废弃物、森林疏伐废弃物、短期轮种木本作物、工业废弃物、小麦秸、燕麦秸、水稻秸、大麦秸、黑麦秸、亚麻秸、大豆壳、稻壳、水稻秸、玉米谷蛋白饲料、燕麦壳、甘蔗、玉米秸秆、玉米杆、玉米芯、玉米壳、柳枝稷、芒草、甜高粱、芸苔茎、大豆茎、草原禾草、鸭茅状摩擦禾、狐尾草;甜菜粕、柑橘果实浆、种子壳、纤维素动物粪便、草坪修剪废弃物、棉花、海藻、树木、软木材、硬木材、白杨、松树、灌木丛、草、小麦、小麦秸、甘蔗渣、玉米、玉米壳、玉米棒、玉米粒、来自玉米粒的纤维、来自谷物湿磨或干磨的产物和副产物、城市固体废弃物、废纸、庭院废弃物、草本材料、农业残余物、林业残余物、城市固体废弃物、废纸、纸浆、造纸厂残余物、树枝、灌木、甘蔗、玉米、玉米壳、能源作物、森林、水果、花、谷物、草、草本作物、树叶、树皮、针叶、原木、根、树苗、灌木丛、柳枝稷,树木、蔬菜、水果皮、藤蔓、甜菜粕、小麦麸皮、燕麦壳、硬木材或软木材、由农业加工产生的有机废弃物材料,林业木材废弃物,或它们中的任意两种或更多种的组合。木质纤维素可被认为是潜在的可再生原料,其通常包括多糖纤维素(葡聚糖)和半纤维素(木聚糖、杂木聚糖和木葡聚糖)。另外,一些半纤维素可以作为葡甘露聚糖存在,例如在木材来源的原料中。这些多糖成为可溶性糖(包括单体和多聚体,例如葡萄糖、纤维二糖、木糖、阿拉伯糖、半乳糖、果糖、甘露糖、鼠李糖、核糖、半乳糖醛酸、葡糖醛酸和其他己糖和戊糖)的酶促水解发生于共同作用的不同酶的作用下。此外,果胶和诸如阿拉伯聚糖的其他果胶物质可占来自非木本植物组织的典型细胞壁干物质的可观的比例(干物质的约四分之一到一半可为果胶)。可以预处理木质纤维素材料。预处理可包括将木质纤维素材料暴露于酸、碱、溶剂、热、过氧化物、臭氧、机械粉碎、碾磨、研磨或快速卸压,或它们中的任意两种或更多种的组合。化学预处理通常与热预处理(例如在150-220℃之间1到30分钟)组合。

[0084] 在另一个实施方式中,所述组合物是淀粉水解产物,诸如玉米淀粉水解产物。

[0085] 在本发明的上下文中,“水解产物”是指多糖已经通过加入水而解聚形成单糖和寡糖。水解产物可以通过含多糖材料的酶促水解或酸水解来产生。

[0086] “表达”是指将基因转录成结构RNA(rRNA、tRNA)或信使RNA(mRNA),随后翻译成蛋白质。

[0087] 如本文所用,“启动子”是指导(结构)基因转录的DNA序列,特别是一种或多种磷酸核酮糖激酶基因。启动子能够使得在厌氧条件期间比在有氧条件下实现更高的表达。

[0088] 除非另有说明,否则本文中不使用数量词修饰时被定义为“至少一个(种)”。

[0089] 当提及名词(例如化合物、添加剂等)而不使用数量词时,意味着包括复数。因此,当提及例如“核苷酸”的特定部分时,除非另有说明,否则这意味着“至少一种”该部分,例如“至少一种核苷酸”。如本文所用的术语“或”或“或者”应理解为“和/或”。

[0090] 术语“发酵”、“发酵性”等在本文中以经典意义使用,即表示工艺在厌氧条件下进行或已经在厌氧条件下进行。厌氧条件在本文中被定义为没有任何氧的条件或酵母细胞(特别是酵母细胞)基本上不消耗氧的条件,并且往往对应于小于5mmol/l.h的氧消耗,特别是小于2.5mmol/l.h或小于1mmol/l.h的氧消耗。更优选地,消耗0mmol/L/h(即,氧消耗为不可检测的)。这往往对应于培养物发酵液中的溶氧浓度小于空气饱和度的5%,特别地溶氧浓度小于空气饱和度的1%或小于空气饱和度的0.2%。

[0091] 术语“酵母”或“酵母细胞”是指一组系统发生上不同的单细胞真菌,其中大多数为子囊菌门(Ascomycota)和担子菌门(Basidiomycota)。芽殖酵母(“真酵母”)被分类为酵母(Saccharomycetales)目,其中酿酒酵母是最众所周知的物种。

[0092] 如本文所用的术语“重组”是指含有这样的核酸的细胞,所述核酸是使用重组DNA技术和/或另一种诱变技术进行一种或多种遗传修饰的结果。特别地,重组细胞可包含不存在于对应野生型细胞中的核酸,该核酸已经使用重组DNA技术引入到该菌株(细胞)中(转基因细胞),或者不存在于所述野生型中的该核酸是在所述野生型中存在的核酸序列(诸如编码野生型多肽的基因)中一例如使用重组DNA技术或另一种诱变技术(诸如UV辐射)一进行一种或多种突变的结果,或者其中基因的核酸序列已被修饰以将多肽产物(编码所述多肽产物)靶向另一个细胞区室。此外,术语“重组(细胞)”特别地涉及这样的菌株(细胞),已经使用重组DNA技术从所述菌株(细胞)中去除了DNA序列。

[0093] 本文所用的术语“核酸”包括对单链或双链形式的脱氧核糖核苷酸或核糖核苷酸的聚合物(即多核苷酸)的提及,并且除非另有限制,否则涵盖具有天然核苷酸的基本性质的已知类似物,即所述已知类似物以类似于天然存在的核苷酸的方式与单链核酸杂交(例如肽核酸)。多核苷酸可以是天然或异源的结构或调控基因的全长序列或子序列。除非另有说明,否则该术语包括对指定序列及其互补序列的提及。因此,具有出于稳定性或其他原因而被修饰的主链的DNA或RNA是“多核苷酸”,正如该术语在本文中所用的。此外,仅举两个示例,包含不常见碱基(诸如肌苷)或经修饰的碱基(诸如三苯甲基化碱基)的DNA或RNA是如本文所使用的术语的多核苷酸。应当理解,已经对DNA和RNA进行了多种修饰,该多种修饰用于本领域技术人员已知的许多有用目的。本文采用的术语多核苷酸包括这些化学、酶促或代谢修饰形式的多核苷酸,以及病毒和细胞(尤其包括简单细胞和复杂细胞)特有的DNA和RNA的化学形式。

[0094] 当参照酶分类(EC)提及酶时,该酶分类是基于由国际生物化学与分子生物学联合会命名委员会(Nomenclature Committee of the International Union of Biochemistry and Molecular Biology,NC-IUBMB)提供的酶命名法,酶被分类为或可分类为的类别,该命名法可在<http://www.chem.qmul.ac.uk/iubmb/enzyme/>处找到。还意于包括没有(尚未)被分类为特定类别但可如此分类的其他合适的酶。

[0095] 如果在本文中通过引用登录号来提及蛋白质或核酸序列(诸如基因),则除非另有说明,否则该数字特别地用于指代具有可经由www.ncbi.nlm.nih.gov/(在2016年6月14日可得的)找到的序列的蛋白质或核酸序列(基因)。

[0096] 如本文所用,具有特定序列(例如“SEQ ID NO:X”)的多肽的术语“功能同源物”(或简称“同源物”)是指包含所述特定序列的多肽,前提条件是一个或多个氨基酸被替换、缺失、添加和/或插入,并且该多肽具有(定性地)用于底物转化的相同酶功能。该功能可以通过使用包含重组酵母细胞的测定系统来测试,该重组细胞包含用于在酵母中表达同源物的表达载体,所述表达载体包含可操作地连接到酵母中的功能性启动子的异源核酸序列,并且所述异源核酸序列编码在酵母细胞中的酶促活性待测试的同源多肽,以及评定所述转化是否发生在所述细胞中。候选同源物可以通过使用计算机相似性分析来鉴定。在W02009/013159的实施例2中描述了这种分析的详细示例。技术人员将能够由此推导出如何可以找到合适的候选同源物,并且任选地在密码子(对)优化后,将能够使用如上所述的合适的测定系统来测试此类候选同源物所需的功能。合适的同源物代表与特定多肽具有大于50%,优选60%或更高,特别地至少70%,更特别地至少80%、至少90%、至少95%、至少97%、至少98%或至少99%的氨基酸序列相似性并具有所需的酶功能的多肽。对于核酸序列,术语功能同源物意于包括由于遗传密码子的简并性而与另一核酸序列不同但编码相同多肽序列的核酸序列。

[0097] 序列同一性在本文中定义为通过比较序列所确定的两个或更多个氨基酸(多肽或蛋白质)序列或两个或更多个核酸(多核苷酸)序列之间的关系。通常,在所比较的序列的全长上比较序列同一性或相似性。在本领域中,“同一性”还意指通过所述序列的链(string)之间的匹配所确定的氨基酸或核酸序列(视情况而定)之间的序列相关性程度。

[0098] 氨基酸或核苷酸序列当表现出一定的相似性水平时,被认为是同源的。同源的两个序列表示共同的进化起源。两个同源序列是密切相关还是更远距离相关由“百分比同一性”或“百分比相似性”表示,“百分比同一性”或“百分比相似性”分别为相关高或低。尽管存在争议,但为了表示“百分比同一性”或“百分比相似性”,“同源性水平”或“百分比同源性”经常互换使用。可以使用数学算法来完成序列的比较和对两个序列之间的百分比同一性的确定。本领域技术人员将意识到以下事实:几种不同的计算机程序可用于比对两个序列并确定两个序列之间的同源性(Kruskal,J.B.(1983) An overview of sequence comparison,在D.Sankoff和J.B.Kruskal(编辑),Time warps,string edits and macromolecules:the theory and practice of sequence comparison,第1-44页,Addison Wesley中)。两个氨基酸序列之间的百分比同一性可以使用用于比对两个序列的Needleman和Wunsch算法来确定。(Needleman,S.B.和Wunsch,C.D.(1970) J.Mol.Biol.48,443-453)。该算法比对氨基酸序列以及核苷酸序列。Needleman-Wunsch算法已在计算机程序NEEDLE中实现。出于本发明的目的,使用来自EMBOSS程序包的NEEDLE程序(2.8.0版或更高版本,EMBOSS:The European Molecular Biology Open Software Suite(2000)Rice,P.Longden,I.和Bleasby,A.Trends in Genetics 16,(6),第276-277页,<http://emboss.bioinformatics.nl/>)。对于蛋白质序列,使用EBL0SUM62来用于替换矩阵。对于核苷酸序列,使用EDNAFULL。可以指定其他矩阵。用于比对氨基酸序列的任选参数是空位开放罚分10和空位延伸罚分0.5。技术人员将理解,所有这些不同的参数将产生略微不同的结果,但是当使用不同的算法时两个序列的总体百分比同一性不会显著改变。

[0099] 全局同一性定义

[0100] 同源性或同一性是在包括任何空位或延伸的整个比对区域上两个完整序列之间

相同匹配的百分比。两个比对序列之间的同源性或同一性计算如下：两个序列在比对中显示出相同氨基酸的对应位置的数目除以包括空位的总比对长度。如本文所定义的同一性可以从NEEDLE获得，并在程序的输出中标记为“同一性 (IDENTITY)”。

[0101] 最长同一性定义

[0102] 两个比对序列之间的同源性或同一性计算如下：两个序列在比对中显示出相同氨基酸的对应位置的数目除以减去比对中的空位总数后的总比对长度。如本文所定义的同一性可以通过使用NOBRIEF选项从NEEDLE获得，并在程序的输出中标记为“最长同一性”。

[0103] 本文所公开的核苷酸或氨基酸序列的变体还可以定义为与 (例如，在序列表中) 本文特别公开的核苷酸或氨基酸序列相比具有一个或若干个替换、插入和/或缺失的核苷酸或氨基酸序列。

[0104] 任选地，在确定氨基酸相似性程度时，技术人员还可以考虑所谓的“保守性”氨基酸替换，如本领域技术人员所清楚的。保守氨基酸替换是指具有相似侧链的残基的可互换性。例如，一组具有脂肪族侧链的氨基酸是甘氨酸、丙氨酸、缬氨酸、亮氨酸和异亮氨酸；一组具有脂肪族-羟基侧链的氨基酸是丝氨酸和苏氨酸；一组具有含酰胺侧链的氨基酸是天冬酰胺和谷氨酰胺；一组具有芳族侧链的氨基酸是苯丙氨酸、酪氨酸和色氨酸；一组具有碱性侧链的氨基酸是赖氨酸、精氨酸和组氨酸；以及一组具有含硫侧链的氨基酸是半胱氨酸和甲硫氨酸。在一个实施方式中，保守氨基酸替换的组是：缬氨酸-亮氨酸-异亮氨酸、苯丙氨酸-酪氨酸、赖氨酸-精氨酸、丙氨酸-缬氨酸，以及天冬酰胺-谷氨酰胺。本文公开的氨基酸序列的替换变体是这样的变体，在所述变体中已去除了所公开序列中的至少一个残基并在该至少一个残基的位置插入不同残基。优选地，氨基酸变化是保守性的。在一个实施方式中，每种天然存在的氨基酸的保守替换如下：Ala至Ser；Arg至Lys；Asn至Gln或His；Asp至Glu；Cys至Ser或Ala；Gln至Asn；Glu至Asp；Gly至Pro；His至Asn或Gln；Ile至Leu或Val；Leu至Ile或Val；Lys至Arg；Gln或Glu；Met至Leu或Ile；Phe至Met、leu或Tyr；Ser至Thr；Thr至Ser；Trp至Tyr；Tyr至Trp或Phe；以及Val至Ile或Leu。

[0105] 核苷酸序列还可以按照它们分别在中等杂交条件下或优选在严格杂交条件下与本文公开的特定核苷酸序列的部分杂交的能力来定义。严格杂交条件在本文中被定义为这样的条件：其允许具有至少约25个核苷酸，优选约50个核苷酸、75个或100个核苷酸，以及最优选约200个或更多个核苷酸的核酸序列在约65℃的温度下在包含约1M盐 (优选6×SSC) 的溶液或具有相当离子强度的任何其他溶液中进行杂交，并在65℃下以包含约0.1M或更少的盐 (优选0.2×SSC) 的溶液或具有相当离子强度的任何其他溶液洗涤。优选地，执行杂交过夜，即至少10小时，并且优选地执行洗涤至少一小时，其中洗涤溶液至少更换两次。这些条件将通常允许具有约90%或更高序列同一性的序列的特异性杂交。

[0106] 中等条件在本文中定义为这样的条件：其允许具有至少50个核苷酸，优选约200个或更多个核苷酸的核酸序列在约45℃的温度下在包含约1M盐 (优选6×SSC) 的溶液或具有相当离子强度的任何其他溶液中杂交，并在室温下以包含约1M盐 (优选6×SSC) 或具有相当离子强度的任何其他溶液洗涤。优选地，执行杂交过夜，即至少10小时，并且优选地执行洗涤至少一小时，其中洗涤溶液至少更换两次。这些条件将通常允许具有高达50%序列同一性的序列的特异性杂交。本领域技术人员将能够修改这些杂交条件，以特异性地鉴定同一性在50%和90%之间变化的序列。

[0107] 如本文所用,关于核酸或蛋白质的“异源”是源自外来物种的核酸或蛋白质,或者如果来自相同物种,则为通过刻意的人为干预而在组成和/或基因组基因座方面被从其天然形式实质性修饰的。例如,与异源结构基因可操作地连接的启动子来自与该结构基因所来源的物种不同的物种,或者如果来自相同物种,则它们中的一者或两者被从其原始形式实质性修饰。异源蛋白质可以源自外来物种,或者如果来自相同物种,则为通过刻意的人为干预而被从其原始形式实质性修饰。

[0108] 重组酵母细胞优选地选自以下项的组:酵母科(Saccharomycetaceae),诸如酿酒酵母、巴氏酵母(*Saccharomyces pastorianus*)、*Saccharomyces beticus*、发酵性酵母(*Saccharomyces fermentati*)、奇异酵母(*Saccharomyces paradoxus*)、葡萄汁酵母(*Saccharomyces uvarum*)和贝酵母(*Saccharomyces bayanus*);裂殖酵母属(*Schizosaccharomyces*),诸如粟酒裂殖酵母(*Schizosaccharomyces pombe*)、日本裂殖酵母(*Schizosaccharomyces japonicus*)、八孢裂殖酵母(*Schizosaccharomyces octosporus*)和嗜冷裂殖酵母(*Schizosaccharomyces cryophilus*);孢圆酵母属(*Torulaspora*),诸如戴尔凯氏孢圆酵母(*Torulaspora delbrueckii*);克鲁维酵母属(*Kluyveromyces*),诸如马克斯克鲁维酵母(*Kluyveromyces marxianus*);毕赤酵母属(*Pichia*),诸如树干毕赤酵母(*Pichia stipitis*)、巴斯德毕赤酵母(*Pichia pastoris*)或安格斯毕赤酵母(*pichia angusta*);接合酵母属(*Zygosaccharomyces*),诸如拜氏接合酵母(*Zygosaccharomyces bailii*);酒香酵母属(*Brettanomyces*),诸如间型酒香酵母(*Brettanomyces intermedius*)、*Brettanomyces bruxellensis*、异酒香酵母(*Brettanomyces anomalus*)、班图酒香酵母(*Brettanomyces custersianus*)、纳氏酒香酵母(*Brettanomyces naardenensis*)、南斯酒香酵母(*Brettanomyces nanus*)、*Dekkera Bruxellis*和异型德克酵母(*Dekkera anomala*);梅奇酵母属(*Metschnikowia*);伊萨酵母属(*Issatchenkia*),诸如东方伊萨酵母(*Issatchenkia orientalis*);克勒克酵母属(*Kloeckera*),诸如柠檬克勒克酵母(*Kloeckera apiculata*);短梗霉属(*Aureobasidium*),诸如出芽短梗霉菌(*Aureobasidium pullulans*)。优选的酵母是酿酒酵母。

[0109] 所述方法中使用的重组酵母可包括:

[0110] -由任选地在强组成型启动子的控制下的PPP基因TAL1、TKL1、RPE1和RKI1组成的组;

[0111] -由在强组成型启动子控制下的xy1A基因组成的组;

[0112] -包含在强组成型启动子控制下的XKS1基因的组,

[0113] -由在强组成型启动子控制下的基因araA、araB和araD组成的组

[0114] -缺失醛糖还原酶基因。

[0115] 在一个实施方式中,重组酵母包含编码醛脱氢酶(E.C.1.2.1.4)的一种或多种内源核苷酸序列的缺失或破坏,或者所述酵母具有与其对应的野生型酵母相比降低的醛脱氢酶活性。

[0116] 如本文所用,醛脱氢酶至少催化以下反应:

[0117] (VII) 乙醛+NADP⁺+H₂O→乙酸+NADPH+H⁺

[0118] 编码醛脱氢酶的所述一种或多种核苷酸序列优选地编码醛脱氢酶ALD2、ALD3、ALD4、ALD5、或ALD6、或具有根据SEQ ID NO:16、SEQ ID NO:27、SEQ ID NO:28、SEQ ID NO:

29或SEQ ID NO:30的氨基酸序列的酶,或具有至少50%,优选地至少60%,更优选地至少70%、75%、80%、85%、90%或95%的序列同一性的其功能同源物,或通过一个或多个氨基酸替换、缺失或插入从SEQ ID NO:16、SEQ ID NO:27、SEQ ID NO:28、SEQ ID NO:29或SEQ ID NO:30的氨基酸序列得到的功能同源物。

[0119] 此类酵母还可以进一步包含:

[0120] -编码具有磷酸转酮酶 (PKL) 活性的酶 (EC 4.1.2.9或EC 4.1.2.22) 或具有根据SEQ ID NO:16、SEQ ID NO:17、SEQ ID NO:18或SEQ ID NO:19的氨基酸序列的酶或具有至少50%,优选地至少60%,更优选地至少70%、75%、80%、85%、90%或95%的序列同一性的其功能同源物,或通过一个或多个氨基酸替换、缺失或插入从SEQ ID NO:16、SEQ ID NO:17、SEQ ID NO:18或SEQ ID NO:19的氨基酸序列得到的功能同源物的一种或多种基因;

[0121] -编码具有磷酸转乙酰酶 (PTA) 活性的酶 (EC 2.3.1.8) 或具有根据SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO:22或SEQ ID NO:23的氨基酸序列的酶或具有至少50%,优选地至少60%,更优选地至少70%、75%、80%、85%、90%或95%的序列同一性的其功能同源物,或通过一个或多个氨基酸替换、缺失或插入从SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO:22或SEQ ID NO:23的氨基酸序列得到的功能同源物的一种或多种基因;和/或

[0122] -编码具有乙酸激酶 (ACK) 活性的酶 (EC 2.7.2.12) 或具有根据SEQ ID NO:24或SEQ ID NO:25的氨基酸序列的酶或具有至少50%,优选地至少60%,更优选地至少70%、75%、80%、85%、90%或95%的序列同一性的其功能同源物,或通过一个或多个氨基酸替换、缺失或插入从SEQ ID NO:24或SEQ ID NO:25的氨基酸序列得到的功能同源物的一种或多种基因。

[0123] 如本文所用,磷酸转酮酶至少催化D-木酮糖5-磷酸转化为D-甘油醛3-磷酸和乙酰磷酸。磷酸转酮酶参与以下反应中的至少一种:

[0124] EC 4.1.2.9:

[0125] (VIII) D-木酮糖-5-磷酸+磷酸 $\rightleftharpoons$ 乙酰磷酸+ D-甘油醛 3-磷酸+H₂O

[0126] D-核酮糖-5-磷酸+磷酸 $\rightleftharpoons$ 乙酰磷酸+ D-甘油醛 3-磷酸+H₂O

[0127] EC 4.1.2.22:

[0128] (IX) D-果糖 6-磷酸+磷酸 $\rightleftharpoons$ 乙酰磷酸+D-赤藓糖 4-磷酸+H₂O

[0129] 用于测量磷酸转酮酶活性的合适的酶测定描述于例如Sonderegger等人(2004, Applied&Environmental Microbiology, 70 (5), 第2892-2897页)中。在一个实施方式中,编码具有磷酸转酮酶活性的酶的一种或多种基因编码具有根据SEQ ID NO:5、SEQ ID NO:6、SEQ ID NO:7或SEQ ID NO:8的氨基酸序列的酶或具有至少50%,优选地至少60%、70%、75%、80%、85%、90%或95%的序列同一性的其功能同源物。编码具有磷酸转酮酶活性的酶的合适核酸序列可以在选自以下组的生物中发现:黑曲霉 (*Aspergillus niger*)、粗糙脉孢菌 (*Neurospora crassa*)、干酪乳杆菌 (*L.casei*)、植物乳杆菌 (*L.plantarum*)、植物乳杆菌、青春双歧杆菌 (*B.adolescentis*)、两歧双歧杆菌 (*B.bifidum*)、高卢双歧杆菌 (*B.gallicum*)、动物双歧杆菌 (*B.animalis*)、乳双歧杆菌 (*B.lactis*)、戊糖乳杆菌 (*L.pentosum*)、嗜酸乳杆菌 (*L.acidophilus*)、产黄青霉 (*P.chrysogenum*)、构巢曲霉 (*A.nidulans*)、棒曲霉 (*A.clavatus*)、肠膜明串珠菌 (*L.mesenteroides*) 和 *O.oenii*。

[0130] 重组细胞可包含编码具有磷酸转乙酰酶活性的酶的一种或多种(异源)基因。如本文所用,磷酸转乙酰酶至少催化乙酰磷酸转化为乙酰辅酶A。在一个实施方式中,编码具有磷酸转乙酰酶活性的酶的一种或多种基因编码具有根据SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11或SEQ ID NO:12的氨基酸序列的酶或具有至少50%,优选地至少60%、70%、75%、80%、85%、90%或95%的序列同一性的其功能同源物。编码具有磷酸转乙酰酶活性的酶的合适核酸序列可以在选自以下组的生物中发现:青春双歧杆菌、枯草芽孢杆菌(*B.subtilis*)、解纤维梭菌(*C.cellulolyticum*)、植物发酵梭菌(*C.phytofermentans*)、两歧双歧杆菌、动物双歧杆菌、肠膜明串珠菌、植物乳杆菌、嗜热毁丝霉(*M.thermophila*)和*O.oeniis*。

[0131] 如本文所用,磷酸转乙酰酶至少催化乙酰磷酸转化为乙酰辅酶A。编码具有磷酸转乙酰酶活性的酶的合适核酸序列可以在选自以下组的生物中发现:青春双歧杆菌、枯草芽孢杆菌、解纤维梭菌、植物发酵梭菌、两歧双歧杆菌、动物双歧杆菌、肠膜明串珠菌、植物乳杆菌、嗜热毁丝霉和*O.oeniis*。

[0132] 如本文所用,乙酸激酶至少催化乙酸转化为乙酰磷酸。

[0133] 如果所述组合是木质纤维素水解产物,则上述酵母特别有用,并且可以使用已知的重组表达技术来构建。辅因子修饰可以在修饰1)至5)中的任一者之前、同时或之后进行。

[0134] 可以对根据本发明的重组酵母细胞进行进化工程以改善其性质。进化工程过程是已知的过程。进化工程是这样的过程,其中微生物(本文中的重组酵母细胞)的工业相关表型可以通过合理设置的自然选择过程而与特定生长速率和/或对营养物的亲和力相偶联。例如,在Kuijper,M等人,FEMS,Eukaryotic cell Research 5 (2005) 925-934,W02008/041840和W02009/112472中详细描述了进化工程。在进化工程化后,分离得到的戊糖发酵重组酵母细胞。分离可以以任何已知的方式进行,例如通过从进化工程中使用的重组酵母细胞发酵液中分离细胞,例如通过采集细胞样品或通过过滤或离心。

[0135] 在一个实施方式中,重组酵母细胞不含标记物。如本文所用,术语“标记物”是指编码允许选择或筛选含有标记物的宿主细胞的性状或表型的基因。不含标记物意指重组酵母细胞中基本上不存在标记物。当抗生素标记物已用于构建重组酵母细胞并随后去除时,不含标记物是特别有利的。可以使用任何合适的现有技术(例如分子内重组)来去除标记物。

[0136] 在一个实施方式中,基于抑制剂耐受性宿主细胞来构建工业重组酵母细胞,其中构建如下文所述进行。可以通过筛选在含抑制剂的材料上生长的菌株来选择抑制剂耐受性宿主细胞,诸如在Kadar等人,Appl.Biochem.Biotechnol.(2007),第136-140卷,847-858中所说明的,其中选择了抑制剂耐受性酿酒酵母菌株ATCC 26602。

[0137] 重组酵母可以包含将丙酮酸转化为期望的发酵产物所需的那些酶促活性,所述期望的发酵产物为诸如乙醇、丁醇(例如正丁醇、2-丁醇和异丁醇)、乳酸、3-羟基-丙酸、丙烯酸、乙酸、琥珀酸、柠檬酸、富马酸、苹果酸、衣康酸、氨基酸、1,3-丙-二醇、乙烯、甘油、 β -内酰胺抗生素或头孢菌素。

[0138] 在一个实施方式中,重组酵母来源于工业重组酵母细胞。工业细胞和工业重组酵母细胞可以如下定义。工业过程中(重组酵母细胞)细胞的生活环境与实验室中的生活环境显著不同。工业重组酵母细胞必须能够在多种环境条件下良好作用,环境条件在该过程中

可能会发生变化。此类变化包括营养源、pH、乙醇浓度、温度、氧浓度等的变化,这些变化一起对酿酒酵母的细胞生长和乙醇生产具有潜在影响。在不利的工业条件下,耐受环境的菌株应允许稳健的生长和生产。工业重组酵母细胞菌株通常对使用它们的应用中可能存在的这些环境条件变化更稳健,所述应用为诸如在烘焙工业、酿造工业、酿酒和生物燃料乙醇工业中。在一个实施方式中,基于工业宿主细胞来构建工业重组酵母细胞,其中构建如下文所述进行。工业酵母细胞(酿酒酵母)的示例是Ethanol Red® (Fermentis)、Fermiol® (DSM)和Thermosacc® (Lallemand)。

[0139] 重组酵母优选地为耐受抑制剂的,即它们可以耐受它们在常见的预处理和水解条件下通常具有的水平的常见抑制剂,使得重组酵母细胞可以具有广泛的应用,即其对于同原料、不同预处理方法和不同水解条件具有高适用性。在一个实施方式中,重组酵母细胞是耐受抑制剂的。抑制剂耐受性是对抑制剂化合物的抵抗力。木质纤维素中抑制性化合物的存在和水平可随原料、预处理方法、水解过程而有很大不同。抑制剂类别的示例是羧酸、呋喃和/或酚类化合物。羧酸的示例是乳酸、乙酸或甲酸。呋喃的示例是糠醛和羟甲基糠醛。酚类化合物的示例是香草醛(vannilin)、丁香酸、阿魏酸和香豆酸。抑制剂的典型量对于羧酸:取决于原料、预处理和水解条件为若干克/升至20克/升或更高。对于呋喃:取决于原料、预处理和水解条件为几百毫克/升到几克/升。对于酚类:取决于原料、预处理和水解条件为几十毫克/升至1克/升。

[0140] 在一个实施方式中,重组酵母细胞是天然能够进行酒精发酵,优选厌氧酒精发酵的细胞。重组酵母细胞优选具有对乙醇的高耐受性、对低pH的高耐受性(即能够在低于约5、约4、约3或约2.5的pH下生长)以及对有机物和/或升高的温度的高耐受性。

[0141] 重组酵母包含在所讨论的细胞中不是天然存在的核苷酸序列,或被用所述核苷酸序列转化或被用所述核苷酸序列遗传修饰。用于在细胞中重组表达酶以及用于重组酵母细胞的其他遗传修饰的技术是本领域技术人员众所周知的。通常,此类技术涉及用包含相关序列的核酸构建体转化细胞。此类方法例如从标准手册(诸如Sambrook和Russel (2001) "Molecular Cloning: A Laboratory Manual (第3版), Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, 或F. Abusable等人编著, "Current protocols in molecular biology", Green Publishing and Wiley Interscience, New York (1987)) 已知的。用于转化和遗传修饰真菌宿主细胞的方法为从例如EP-A-0635574、W098/46772、W099/60102、W000/37671、W090/14423、EP-A-0481008、EP-A-0635574和US6,265,186已知的。

[0142] 生产乙醇的方法是发酵方法,所述发酵方法优选地在对于酵母最佳的温度下进行。因此,对于大多数重组酵母细胞而言,在低于约50°C、低于约42°C、或低于约38°C的温度下执行发酵过程。

[0143] 发酵过程可以以分批、补料分批或连续方式进行。也可以采用单独水解和发酵(SHF)方法或同时糖化和发酵(SSF)方法。这些发酵过程模式的组合也可实现最佳生产率。

[0144] 乙醇的回收是本领域中已知的,并且可以包括分级和吸附技术。例如,可以使用发酵醪蒸馏器(beer still)处理发酵产物(其在含水混合物中含有乙醇),以产生富含乙醇的混合物,然后将该富含乙醇的混合物进行分级(例如,分馏或其他类似技术)。接下来,可以使含有最高浓度的乙醇的级分通过吸附器,以从乙醇中去除大部分(如果不是全部)的剩余水。在一个实施方式中,除了回收发酵产物之外,还可以将酵母再循环。以下非限制性实施

例仅旨在为示例性的。

实施例

[0145] 材料和方法

[0146] 培养基

[0147] 发酵是使用合成培养基 (Luttik 2000) 和工业培养基 (玉米秸秆水解产物、玉米醪液) 执行的。每当施用组氨酸营养缺陷型菌株时, 向培养基中补充200mg/L组氨酸。玉米秸秆水解产物培养基是通过用去离子水将17%ds的玉米秸秆水解产物稀释1.5倍并向其补充24g/L木糖、1g/L尿素以及与水解产物中的乙酸等摩尔量的甘油来制备的。为防止水解产物中存在的任何细菌污染物的过度生长, 将新霉素和青霉素G加入至终浓度分别为50 μ g/ml和100 μ g/ml。加入约250 μ l/l的硅氧烷消泡剂 (Dow Corning 1520) 以防止起泡。预先在NREL上对所施加的水解产物进行稀酸预处理, 使用2M氨将pH (从大致1-2) 调节至pH 4.5, 然后使用DSM专有的酶混合物进行酶促水解。发酵之前, 用2M KOH将培养基的pH调节至5.5。

[0148] 通过以下方式制备玉米醪液: 将30%w/w的粉碎的玉米固体 (Limagrain Westhove 玉蜀黍L3) 与脱矿质水混合, 用2M H₂SO₄将pH调节至5.5, 加入0.02%w/w的 α -淀粉酶 (Termamyl, Novozymes), 以及在旋转摇床 (150RPM) 中在80 $^{\circ}$ C下孵育4小时。加入尿素 (1.25g/L) 作为N源。为了模拟玉米乙醇厂中发现的乙酸浓度, 向醪液中加入1.5g/L乙酸, 并使用2M H₂SO₄/KOH将pH调节至5.0。发酵开始时, 加入0.16g/kg的葡糖淀粉酶 (Spirizyme, Novozymes)。

[0149] 预培养条件

[0150] 将一接种环的冷冻的 (甘油) 贮存培养物在YhPD (10g/l酵母提取物、20g/l的植物蛋白胨、20g/l葡萄糖和15g/l琼脂) 平板上划线, 并在30 $^{\circ}$ C下孵育3天。将3个菌落从琼脂平板转移到500ml摇瓶中, 所述摇瓶中装有200ml矿物培养基 (Luttik等人, 2000), 用6N KOH调节至pH 6.0。将培养物在30 $^{\circ}$ C下的振荡培养箱 (200RPM) 中孵育过夜 (17-20小时)。在通过过滤确定培养物的干细胞重量 (DCW) 含量 (参见干细胞重量测定) 之后。将与发酵所需接种量对应的一定量的预培养物离心 (3min, 13500x g), 用一个培养体积的冷 (4 $^{\circ}$ C) 无菌脱矿质水洗涤一次, 再离心一次, 重悬于发酵培养基中, 并转移至发酵罐中。

[0151] 发酵条件

[0152] 发酵是在DASbox (Eppendorf, Hamburg, Germany) 250ml微型生物反应器系统中以200ml工作体积执行的。将发酵温度控制在32 $^{\circ}$ C, 以300RPM搅拌, 并且在需要时, 通过控制加入2M H₂SO₄将pH限制在指定的上限值。为了确保厌氧条件, 顶部空间喷流50ml/min的N₂。

[0153] 菌株

[0154] 菌株YD01437已经在PCT/EP2014/068325 $\rightarrow$ W02015/028583中详细描述, 特别是在实施例6和实施例7中的菌株T5。

[0155] 样品分析

[0156] 通过以下方式测定干细胞重量: 在预先称重的硝化纤维素滤器 (孔径为0.45mm) 上过滤已知量的发酵液样品, 并用脱矿质水洗涤酵母生物质, 在103 $^{\circ}$ C下干燥4小时并称重。离心后, 通过对发酵液样品的上清液进行HPLC分析来确定发酵液中的代谢产物。在W02016/097202中详细描述了所应用的方法。对于SSF样品, 将1mL/L的10g/L阿卡波糖贮液加入到样

品中以抑制葡糖淀粉酶活性。

[0157] 实施例1

[0158] 合成培养基

[0159] 合成培养基 (luttik 2000), 含有10% m/v 葡萄糖、1.5g/L 乙酸, 用2M KOH 设定 (起始) pH 为5.5。投加0.5g的CDW/L YD01437。

[0160] 无pH控制 (A)

[0161] 乙酸随着菌株厌氧生长而被转化, pH 升高至约7.0, 此时尽管存在约0.54g/L 的总乙酸, 但是由于达到了未离解的乙酸的最低水平, 乙酸的转化停止。因此, 酵母的生长停止或者非常缓慢。

[0162] 将pH维持在5.5 (B)

[0163] 乙酸随着菌株厌氧生长而被转化, 但pH 值维持在5.5。因此, 乙酸的转化和生长持续更长的时间 (与A相比), 直到在葡萄糖耗尽之前, 乙酸也达到了约50 μ M 未离解的乙酸的最低阈值。现在, 剩余的乙酸总量要低得多: 约0.001g/L, 从而导致了更高的乙醇产率。

[0164] 实施例2

[0165] 木质纤维素水解产物

[0166] 玉米秸秆水解产物培养基, 投加0.5g的CDW/L YD01437。

[0167] 无pH控制 (C)

[0168] 乙酸被转化 (与甘油共转化), pH 升高至7.1, 此时尽管存在约2.5g/L 的总乙酸, 但由于达到了约50 μ M 的未离解的乙酸的最低阈值, 乙酸转化停止。糖 (木糖) 转化一直持续直到耗尽。

[0169] 将pH维持在5.5 (D)

[0170] 乙酸被转化 (与甘油共转化), 但pH 保持在5.5, 因此乙酸转化持续更长时间 (与C相比), 直至木糖耗尽之前, 乙酸也达到了约50 μ M 未离解的乙酸的最低阈值。现在, 剩余的乙酸总量要低得多: 约0.02g/L, 从而导致了更高的乙醇产率。

[0171] 实施例3

[0172] 玉米醪液

[0173] 玉米醪液, 投加0.075g的CDW/L YD01437

[0174] 无pH控制 (C)

[0175] 乙酸随着菌株厌氧生长而被转化, pH 升高至7.0, 此时尽管存在残留量的总乙酸, 但由于达到了约50 μ M 的未离解的乙酸的最低阈值, 乙酸转化停止。由于缺乏氧化还原池, 酵母生长停止或非常缓慢。游离葡萄糖将由于缺乏生长而积聚, 从而给酵母带来渗透应力 (osmostress)。葡萄糖转化以逐渐降低的速率进行, 或者可完全停止。

[0176] 将pH维持在5.5 (D)

[0177] 乙酸随着菌株厌氧生长而被转化, 但pH 保持在5.5, 因此乙酸转化持续更长的时间 (与C相比), 直到在葡萄糖耗尽之前, 乙酸也达到了约50 μ M 未离解的乙酸的最低阈值。现在, 剩余的乙酸总量要低得多: 约0.001g/L, 从而导致了更高的乙醇产率。

序列表

<110> 帝斯曼知识产权资产管理有限公司

<120> 消耗乙酸的菌株

<130> 32574-W0-PCT

<160> 30

<170> BiSSAP 1.3.6

<210> 1

<211> 891

<212> PRT

<213> 大肠杆菌 (Escherichia coli)

<220>

<223> 大肠杆菌双功能NAD+依赖性乙酰化

乙醛/醇脱氢酶(大肠杆菌adhE)氨基酸

序列

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20 25 30

Lys Ile Phe Arg Ala Ala Ala Leu Ala Ala Ala Asp Ala Arg Ile Pro
35 40 45

Leu Ala Lys Met Ala Val Ala Glu Ser Gly Met Gly Ile Val Glu Asp
50 55 60

Lys Val Ile Lys Asn His Phe Ala Ser Glu Tyr Ile Tyr Asn Ala Tyr
65 70 75 80

Lys Asp Glu Lys Thr Cys Gly Val Leu Ser Glu Asp Asp Thr Phe Gly
85 90 95

Thr Ile Thr Ile Ala Glu Pro Ile Gly Ile Ile Cys Gly Ile Val Pro
100 105 110

Thr Thr Asn Pro Thr Ser Thr Ala Ile Phe Lys Ser Leu Ile Ser Leu
115 120 125

Lys Thr Arg Asn Ala Ile Ile Phe Ser Pro His Pro Arg Ala Lys Asp
130 135 140

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

Gly Ala Pro Lys Asp Leu Ile Gly Trp Ile Asp Gln Pro Ser Val Glu
165 170 175

Leu Ser Asn Ala Leu Met His His Pro Asp Ile Asn Leu Ile Leu Ala

180	185	190
Thr Gly Gly Pro Gly Met Val Lys	Ala Ala Tyr Ser Ser Gly Lys Pro	
195	200	205
Ala Ile Gly Val Gly Ala Gly Asn Thr Pro Val Val Ile Asp Glu Thr		
210	215	220
Ala Asp Ile Lys Arg Ala Val Ala Ser Val Leu Met Ser Lys Thr Phe		
225	230	235
Asp Asn Gly Val Ile Cys Ala Ser Glu Gln Ser Val Val Val Val Asp		
245	250	255
Ser Val Tyr Asp Ala Val Arg Glu Arg Phe Ala Thr His Gly Gly Tyr		
260	265	270
Leu Leu Gln Gly Lys Glu Leu Lys Ala Val Gln Asp Val Ile Leu Lys		
275	280	285
Asn Gly Ala Leu Asn Ala Ala Ile Val Gly Gln Pro Ala Tyr Lys Ile		
290	295	300
Ala Glu Leu Ala Gly Phe Ser Val Pro Glu Asn Thr Lys Ile Leu Ile		
305	310	315
Gly Glu Val Thr Val Val Asp Glu Ser Glu Pro Phe Ala His Glu Lys		
325	330	335
Leu Ser Pro Thr Leu Ala Met Tyr Arg Ala Lys Asp Phe Glu Asp Ala		
340	345	350
Val Glu Lys Ala Glu Lys Leu Val Ala Met Gly Gly Ile Gly His Thr		
355	360	365
Ser Cys Leu Tyr Thr Asp Gln Asp Asn Gln Pro Ala Arg Val Ser Tyr		
370	375	380
Phe Gly Gln Lys Met Lys Thr Ala Arg Ile Leu Ile Asn Thr Pro Ala		
385	390	395
Ser Gln Gly Gly Ile Gly Asp Leu Tyr Asn Phe Lys Leu Ala Pro Ser		
405	410	415
Leu Thr Leu Gly Cys Gly Ser Trp Gly Gly Asn Ser Ile Ser Glu Asn		
420	425	430
Val Gly Pro Lys His Leu Ile Asn Lys Lys Thr Val Ala Lys Arg Ala		
435	440	445
Glu Asn Met Leu Trp His Lys Leu Pro Lys Ser Ile Tyr Phe Arg Arg		
450	455	460
Gly Ser Leu Pro Ile Ala Leu Asp Glu Val Ile Thr Asp Gly His Lys		
465	470	475
Arg Ala Leu Ile Val Thr Asp Arg Phe Leu Phe Asn Asn Gly Tyr Ala		
485	490	495

Asp Gln Ile Thr Ser Val Leu Lys Ala Ala Gly Val Glu Thr Glu Val		
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Phe Phe Glu Val Glu Ala Asp Pro Thr Leu Ser Ile Val Arg Lys Gly		
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Ala Glu Leu Ala Asn Ser Phe Lys Pro Asp Val Ile Ile Ala Leu Gly		
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Gly Gly Ser Pro Met Asp Ala Ala Lys Ile Met Trp Val Met Tyr Glu		
545	550	555
His Pro Glu Thr His Phe Glu Glu Leu Ala Leu Arg Phe Met Asp Ile		
565	570	575
Arg Lys Arg Ile Tyr Lys Phe Pro Lys Met Gly Val Lys Ala Lys Met		
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Ile Ala Val Thr Thr Thr Ser Gly Thr Gly Ser Glu Val Thr Pro Phe		
595	600	605
Ala Val Val Thr Asp Asp Ala Thr Gly Gln Lys Tyr Pro Leu Ala Asp		
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Tyr Ala Leu Thr Pro Asp Met Ala Ile Val Asp Ala Asn Leu Val Met		
625	630	635
Asp Met Pro Lys Ser Leu Cys Ala Phe Gly Gly Leu Asp Ala Val Thr		
645	650	655
His Ala Met Glu Ala Tyr Val Ser Val Leu Ala Ser Glu Phe Ser Asp		
660	665	670
Gly Gln Ala Leu Gln Ala Leu Lys Leu Leu Lys Glu Tyr Leu Pro Ala		
675	680	685
Ser Tyr His Glu Gly Ser Lys Asn Pro Val Ala Arg Glu Arg Val His		
690	695	700
Ser Ala Ala Thr Ile Ala Gly Ile Ala Phe Ala Asn Ala Phe Leu Gly		
705	710	715
Val Cys His Ser Met Ala His Lys Leu Gly Ser Gln Phe His Ile Pro		
725	730	735
His Gly Leu Ala Asn Ala Leu Leu Ile Cys Asn Val Ile Arg Tyr Asn		
740	745	750
Ala Asn Asp Asn Pro Thr Lys Gln Thr Ala Phe Ser Gln Tyr Asp Arg		
755	760	765
Pro Gln Ala Arg Arg Arg Tyr Ala Glu Ile Ala Asp His Leu Gly Leu		
770	775	780
Ser Ala Pro Gly Asp Arg Thr Ala Ala Lys Ile Glu Lys Leu Leu Ala		
785	790	795
Trp Leu Glu Thr Leu Lys Ala Glu Leu Gly Ile Pro Lys Ser Ile Arg		

	805		810		815
Glu Ala Gly Val Gln Glu Ala Asp Phe Leu Ala Asn Val Asp Lys Leu					
	820		825		830
Ser Glu Asp Ala Phe Asp Asp Gln Cys Thr Gly Ala Asn Pro Arg Tyr					
	835		840		845
Pro Leu Ile Ser Glu Leu Lys Gln Ile Leu Leu Asp Thr Tyr Tyr Gly					
	850		855		860
Arg Asp Tyr Val Glu Gly Glu Thr Ala Ala Lys Lys Glu Ala Ala Pro					
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Ala Lys Ala Glu Lys Lys Ala Lys Lys Ser Ala					
	885		890		
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<213> 大肠杆菌(Escherichia coli)					
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Phe Ala Ser Leu Asp Asp Ala Val Ala Ala Ala Lys Val Ala Gln Gln					
	35		40		45
Gly Leu Lys Ser Val Ala Met Arg Gln Leu Ala Ile Ala Ala Ile Arg					
	50		55		60
Glu Ala Gly Glu Lys His Ala Arg Asp Leu Ala Glu Leu Ala Val Ser					
65		70		75	80
Glu Thr Gly Met Gly Arg Val Glu Asp Lys Phe Ala Lys Asn Val Ala					
	85		90		95
Gln Ala Arg Gly Thr Pro Gly Val Glu Cys Leu Ser Pro Gln Val Leu					
	100		105		110
Thr Gly Asp Asn Gly Leu Thr Leu Ile Glu Asn Ala Pro Trp Gly Val					
	115		120		125
Val Ala Ser Val Thr Pro Ser Thr Asn Pro Ala Ala Thr Val Ile Asn					
	130		135		140
Asn Ala Ile Ser Leu Ile Ala Ala Gly Asn Ser Val Ile Phe Ala Pro					
145		150		155	160

His Pro Ala Ala Lys Lys Val Ser Gln Arg Ala Ile Thr Leu Leu Asn		
165	170	175
Gln Ala Ile Val Ala Ala Gly Gly Pro Glu Asn Leu Leu Val Thr Val		
180	185	190
Ala Asn Pro Asp Ile Glu Thr Ala Gln Arg Leu Phe Lys Phe Pro Gly		
195	200	205
Ile Gly Leu Leu Val Val Thr Gly Gly Glu Ala Val Val Glu Ala Ala		
210	215	220
Arg Lys His Thr Asn Lys Arg Leu Ile Ala Ala Gly Ala Gly Asn Pro		
225	230	235
Pro Val Val Val Asp Glu Thr Ala Asp Leu Ala Arg Ala Ala Gln Ser		
245	250	255
Ile Val Lys Gly Ala Ser Phe Asp Asn Asn Ile Ile Cys Ala Asp Glu		
260	265	270
Lys Val Leu Ile Val Val Asp Ser Val Ala Asp Glu Leu Met Arg Leu		
275	280	285
Met Glu Gly Gln His Ala Val Lys Leu Thr Ala Glu Gln Ala Gln Gln		
290	295	300
Leu Gln Pro Val Leu Leu Lys Asn Ile Asp Glu Arg Gly Lys Gly Thr		
305	310	315
Val Ser Arg Asp Trp Val Gly Arg Asp Ala Gly Lys Ile Ala Ala Ala		
325	330	335
Ile Gly Leu Lys Val Pro Gln Glu Thr Arg Leu Leu Phe Val Glu Thr		
340	345	350
Thr Ala Glu His Pro Phe Ala Val Thr Glu Leu Met Met Pro Val Leu		
355	360	365
Pro Val Val Arg Val Ala Asn Val Ala Asp Ala Ile Ala Leu Ala Val		
370	375	380
Lys Leu Glu Gly Gly Cys His His Thr Ala Ala Met His Ser Arg Asn		
385	390	395
Ile Glu Asn Met Asn Gln Met Ala Asn Ala Ile Asp Thr Ser Ile Phe		
405	410	415
Val Lys Asn Gly Pro Cys Ile Ala Gly Leu Gly Leu Gly Gly Glu Gly		
420	425	430
Trp Thr Thr Met Thr Ile Thr Thr Pro Thr Gly Glu Gly Val Thr Ser		
435	440	445
Ala Arg Thr Phe Val Arg Leu Arg Arg Cys Val Leu Val Asp Ala Phe		
450	455	460
Arg Ile Val		

465

<210> 3

<211> 455

<212> PRT

<213> 植物乳杆菌(Lactobacillus plantarum)

<220>

<223> 植物乳杆菌乙醛脱氢酶(Lpla_acdH)氨基酸
序列

<400> 3

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          20           25           30
Gln Ser Gln Val Asp Gln Ala Val Ala Ala Met Ala Arg Ala Ala His
          35           40           45
Ala Ala Arg Gly Met Leu Ala Ala Met Ala Val Glu Glu Thr Gly Arg
          50           55           60
Gly Asn Tyr Arg Asp Lys Val Ala Lys Asn Asp Phe Ala Ala Lys Asn
65           70           75           80
Val Tyr Asn Tyr Ile Lys Asp Asp Lys Thr Val Gly Ile Ile Asn Asp
          85           90           95
Asp Pro Val Ser Gly Val Met Lys Val Ala Glu Pro Val Gly Ile Ile
          100          105          110
Ala Gly Val Thr Pro Val Thr Asn Pro Thr Ser Thr Val Ile Phe Asn
          115          120          125
Ala Met Leu Ala Leu Lys Thr Arg Asn Pro Ile Ile Phe Gly Phe His
          130          135          140
Pro Phe Ala Gln Lys Ser Cys Val Glu Thr Gly Arg Ile Ile Arg Asp
145          150          155          160
Ala Ala Ile Ala Ser Gly Ala Pro Lys Asp Trp Ile Gln Trp Ile Lys
          165          170          175
Thr Pro Ser Leu Glu Ala Thr Asn Thr Leu Met Asn His Pro Gly Val
          180          185          190
Ala Thr Ile Ile Ala Thr Gly Gly Ala Gly Met Val Lys Thr Ala Tyr
          195          200          205
Ser Thr Gly Lys Pro Ala Leu Gly Val Gly Pro Gly Asn Val Pro Cys
          210          215          220
Phe Ile Glu Gln Thr Ala Asp Ile Gln Gln Ala Val Ser Asp Val Val
225          230          235          240

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Thr Ser Lys Ser Phe Asp Asn Gly Met Ile Cys Ala Ser Glu Ser Asn		
245	250	255
Leu Ile Val Ala Asp Gln Ile Tyr Asp Gln Val Lys Arg Glu Leu Ser		
260	265	270
His Asn Gly Val Tyr Phe Val Gly Thr Glu Asn Phe Lys Ala Leu Glu		
275	280	285
Ala Thr Val Met Asn Leu Asp Lys Gln Ala Val Asp Pro Lys Val Ala		
290	295	300
Gly Gln Thr Pro Trp Gln Ile Ala Gln Trp Ala Gly Phe Asp Val Pro		
305	310	315
Ser Asp Thr Lys Val Leu Ala Val Glu Leu Pro Ser Ile Gly Gly Asp		
325	330	335
Gln Val Leu Ser Arg Glu Lys Leu Ser Pro Val Leu Ala Val Val His		
340	345	350
Ala Lys Asp Thr Glu Ala Gly Phe Asn Leu Met Lys Arg Ser Leu Ala		
355	360	365
Leu Gly Gly Leu Gly His Thr Ala Ala Leu His Thr Thr Asp Glu Ala		
370	375	380
Val Met Asn Lys Phe Ala Leu Glu Met Thr Ala Cys Arg Ala Leu Ile		
385	390	395
Asn Val Pro Ser Ser Gln Gly Ala Ile Gly Tyr Lys Tyr Asp Asn Val		
405	410	415
Ala Pro Ser Leu Thr Leu Gly Cys Gly Thr Trp Gly His Asn Ser Ile		
420	425	430
Ser His Asn Leu Glu Asp Trp Asp Leu Leu Asn Ile Lys Thr Val Ala		
435	440	445
Lys Arg Leu Thr Lys Ile Arg		
450	455	
<210> 4		
<211> 469		
<212> PRT		
<213> 无害利斯特菌 (<i>Listeria innocua</i>)		
<220>		
<223> 无害利斯特菌乙醛脱氢酶 (Linn_acdH) 氨基酸		
序列		
<400> 4		
Met Glu Ser Leu Glu Leu Glu Gln Leu Val Lys Lys Val Leu Leu Glu		
1	5	10
Lys Leu Ala Glu Gln Lys Glu Val Pro Thr Lys Thr Thr Thr Gln Gly		

20	25	30
Ala Lys Ser Gly Val Phe Asp Thr	Val Asp Glu Ala Val Gln Ala Ala	
35	40	45
Val Ile Ala Gln Asn Cys Tyr Lys	Glu Lys Ser Leu Glu Glu Arg Arg	
50	55	60
Asn Val Val Lys Ala Ile Arg Glu	Ala Leu Tyr Pro Glu Ile Glu Thr	
65	70	75
Ile Ala Thr Arg Ala Val Ala Glu	Thr Gly Met Gly Asn Val Thr Asp	
85	90	95
Lys Ile Leu Lys Asn Thr Leu Ala	Ile Glu Lys Thr Pro Gly Val Glu	
100	105	110
Asp Leu Tyr Thr Glu Val Ala Thr	Gly Asp Asn Gly Met Thr Leu Tyr	
115	120	125
Glu Leu Ser Pro Tyr Gly Val Ile	Gly Ala Val Ala Pro Ser Thr Asn	
130	135	140
Pro Thr Glu Thr Leu Ile Cys Asn	Ser Ile Gly Met Leu Ala Ala Gly	
145	150	155
Asn Ala Val Phe Tyr Ser Pro His	Pro Gly Ala Lys Asn Ile Ser Leu	
165	170	175
Trp Leu Ile Glu Lys Leu Asn Thr	Ile Val Arg Asp Ser Cys Gly Ile	
180	185	190
Asp Asn Leu Ile Val Thr Val Ala	Lys Pro Ser Ile Gln Ala Ala Gln	
195	200	205
Glu Met Met Asn His Pro Lys Val	Pro Leu Leu Val Ile Thr Gly Gly	
210	215	220
Pro Gly Val Val Leu Gln Ala Met	Gln Ser Gly Lys Lys Val Ile Gly	
225	230	235
Ala Gly Ala Gly Asn Pro Pro Ser	Ile Val Asp Glu Thr Ala Asn Ile	
245	250	255
Glu Lys Ala Ala Ala Asp Ile Val	Asp Gly Ala Ser Phe Asp His Asn	
260	265	270
Ile Leu Cys Ile Ala Glu Lys Ser	Val Val Ala Val Asp Ser Ile Ala	
275	280	285
Asp Phe Leu Leu Phe Gln Met Glu	Lys Asn Gly Ala Leu His Val Thr	
290	295	300
Asn Pro Ser Asp Ile Gln Lys Leu	Glu Lys Val Ala Val Thr Asp Lys	
305	310	315
Gly Val Thr Asn Lys Lys Leu Val	Gly Lys Ser Ala Thr Glu Ile Leu	
325	330	335

Lys Glu Ala Gly Ile Ala Cys Asp Phe Thr Pro Arg Leu Ile Ile Val
 340 345 350
 Glu Thr Glu Lys Ser His Pro Phe Ala Thr Val Glu Leu Leu Met Pro
 355 360 365
 Ile Val Pro Val Val Arg Val Pro Asp Phe Asp Glu Ala Leu Glu Val
 370 375 380
 Ala Ile Glu Leu Glu Gln Gly Leu His His Thr Ala Thr Met His Ser
 385 390 395 400
 Gln Asn Ile Ser Arg Leu Asn Lys Ala Ala Arg Asp Met Gln Thr Ser
 405 410 415
 Ile Phe Val Lys Asn Gly Pro Ser Phe Ala Gly Leu Gly Phe Arg Gly
 420 425 430
 Glu Gly Ser Thr Thr Phe Thr Ile Ala Thr Pro Thr Gly Glu Gly Thr
 435 440 445
 Thr Thr Ala Arg His Phe Ala Arg Arg Arg Arg Cys Val Leu Thr Asp
 450 455 460
 Gly Phe Ser Ile Arg
 465
 <210> 5
 <211> 869
 <212> PRT
 <213> 金黄色酿脓葡萄球菌 (Staphylococcus aureus)
 <220>
 <223> 金黄色酿脓葡萄球菌乙醛/醇脱氢酶 (Saur_adhE) 氨基酸
 序列
 <400> 5
 Met Leu Thr Ile Pro Glu Lys Glu Asn Arg Gly Ser Lys Glu Gln Glu
 1 5 10 15
 Val Ala Ile Met Ile Asp Ala Leu Ala Asp Lys Gly Lys Lys Ala Leu
 20 25 30
 Glu Ala Leu Ser Lys Lys Ser Gln Glu Glu Ile Asp His Ile Val His
 35 40 45
 Gln Met Ser Leu Ala Ala Val Asp Gln His Met Val Leu Ala Lys Leu
 50 55 60
 Ala His Glu Glu Thr Gly Arg Gly Ile Tyr Glu Asp Lys Ala Ile Lys
 65 70 75 80
 Asn Leu Tyr Ala Ser Glu Tyr Ile Trp Asn Ser Ile Lys Asp Asn Lys
 85 90 95
 Thr Val Gly Ile Ile Gly Glu Asp Lys Glu Lys Gly Leu Thr Tyr Val

100	105	110
Ala Glu Pro Ile Gly Val Ile Cys Gly Val Thr Pro Thr Thr Asn Pro		
115	120	125
Thr Ser Thr Thr Ile Phe Lys Ala Met Ile Ala Ile Lys Thr Gly Asn		
130	135	140
Pro Ile Ile Phe Ala Phe His Pro Ser Ala Gln Glu Ser Ser Lys Arg		
145	150	155
Ala Ala Glu Val Val Leu Glu Ala Ala Met Lys Ala Gly Ala Pro Lys		
165	170	175
Asp Ile Ile Gln Trp Ile Glu Val Pro Ser Ile Glu Ala Thr Lys Gln		
180	185	190
Leu Met Asn His Lys Gly Ile Ala Leu Val Leu Ala Thr Gly Gly Ser		
195	200	205
Gly Met Val Lys Ser Ala Tyr Ser Thr Gly Lys Pro Ala Leu Gly Val		
210	215	220
Gly Pro Gly Asn Val Pro Ser Tyr Ile Glu Lys Thr Ala His Ile Lys		
225	230	235
Arg Ala Val Asn Asp Ile Ile Gly Ser Lys Thr Phe Asp Asn Gly Met		
245	250	255
Ile Cys Ala Ser Glu Gln Val Val Val Ile Asp Lys Glu Ile Tyr Lys		
260	265	270
Asp Val Thr Asn Glu Phe Lys Ala His Gln Ala Tyr Phe Val Lys Lys		
275	280	285
Asp Glu Leu Gln Arg Leu Glu Asn Ala Ile Met Asn Glu Gln Lys Thr		
290	295	300
Gly Ile Lys Pro Asp Ile Val Gly Lys Ser Ala Val Glu Ile Ala Glu		
305	310	315
Leu Ala Gly Ile Pro Val Pro Glu Asn Thr Lys Leu Ile Ile Ala Glu		
325	330	335
Ile Ser Gly Val Gly Ser Asp Tyr Pro Leu Ser Arg Glu Lys Leu Ser		
340	345	350
Pro Val Leu Ala Leu Val Lys Ala Gln Ser Thr Lys Gln Ala Phe Gln		
355	360	365
Ile Cys Glu Asp Thr Leu His Phe Gly Gly Leu Gly His Thr Ala Val		
370	375	380
Ile His Thr Glu Asp Glu Thr Leu Gln Lys Asp Phe Gly Leu Arg Met		
385	390	395
Lys Ala Cys Arg Val Leu Val Asn Thr Pro Ser Ala Val Gly Gly Ile		
405	410	415

Gly Asp Met Tyr Asn Glu Leu Ile Pro Ser Leu Thr Leu Gly Cys Gly		
420	425	430
Ser Tyr Gly Arg Asn Ser Ile Ser His Asn Val Ser Ala Thr Asp Leu		
435	440	445
Leu Asn Ile Lys Thr Ile Ala Lys Arg Arg Asn Asn Thr Gln Ile Phe		
450	455	460
Lys Val Pro Ala Gln Ile Tyr Phe Glu Glu Asn Ala Ile Met Ser Leu		
465	470	475
Thr Thr Met Asp Lys Ile Glu Lys Val Met Ile Val Cys Asp Pro Gly		
485	490	495
Met Val Glu Phe Gly Tyr Thr Lys Thr Val Glu Asn Val Leu Arg Gln		
500	505	510
Arg Thr Glu Gln Pro Gln Ile Lys Ile Phe Ser Glu Val Glu Pro Asn		
515	520	525
Pro Ser Thr Asn Thr Val Tyr Lys Gly Leu Glu Met Met Val Asp Phe		
530	535	540
Gln Pro Asp Thr Ile Ile Ala Leu Gly Gly Gly Ser Ala Met Asp Ala		
545	550	555
Ala Lys Ala Met Trp Met Phe Phe Glu His Pro Glu Thr Ser Phe Phe		
565	570	575
Gly Ala Lys Gln Lys Phe Leu Asp Ile Gly Lys Arg Thr Tyr Lys Ile		
580	585	590
Gly Met Pro Glu Asn Ala Thr Phe Ile Cys Ile Pro Thr Thr Ser Gly		
595	600	605
Thr Gly Ser Glu Val Thr Pro Phe Ala Val Ile Thr Asp Ser Glu Thr		
610	615	620
Asn Val Lys Tyr Pro Leu Ala Asp Phe Ala Leu Thr Pro Asp Val Ala		
625	630	635
Ile Ile Asp Pro Gln Phe Val Met Ser Val Pro Lys Ser Val Thr Ala		
645	650	655
Asp Thr Gly Met Asp Val Leu Thr His Ala Met Glu Ser Tyr Val Ser		
660	665	670
Val Met Ala Ser Asp Tyr Thr Arg Gly Leu Ser Leu Gln Ala Ile Lys		
675	680	685
Leu Thr Phe Glu Tyr Leu Lys Ser Ser Val Glu Lys Gly Asp Lys Val		
690	695	700
Ser Arg Glu Lys Met His Asn Ala Ser Thr Leu Ala Gly Met Ala Phe		
705	710	715
Ala Asn Ala Phe Leu Gly Ile Ala His Ser Ile Ala His Lys Ile Gly		

					725						730						735
Gly	Glu	Tyr	Gly	Ile	Pro	His	Gly	Arg	Ala	Asn	Ala	Ile	Leu	Leu	Pro		
					740						745						750
His	Ile	Ile	Arg	Tyr	Asn	Ala	Lys	Asp	Pro	Gln	Lys	His	Ala	Leu	Phe		
					755						760						765
Pro	Lys	Tyr	Glu	Phe	Phe	Arg	Ala	Asp	Thr	Asp	Tyr	Ala	Asp	Ile	Ala		
					770						775						780
Lys	Phe	Leu	Gly	Leu	Lys	Gly	Asn	Thr	Thr	Glu	Ala	Leu	Val	Glu	Ser		
					785						790						800
Leu	Ala	Lys	Ala	Val	Tyr	Glu	Leu	Gly	Gln	Ser	Val	Gly	Ile	Glu	Met		
					805						810						815
Asn	Leu	Lys	Ser	Gln	Gly	Val	Ser	Glu	Glu	Glu	Leu	Asn	Glu	Ser	Ile		
					820						825						830
Asp	Arg	Met	Ala	Glu	Leu	Ala	Phe	Glu	Asp	Gln	Cys	Thr	Thr	Ala	Asn		
					835						840						845
Pro	Lys	Glu	Ala	Leu	Ile	Ser	Glu	Ile	Lys	Asp	Ile	Ile	Gln	Thr	Ser		
					850						855						860
Tyr Asp Tyr Lys Gln																	
865																	
<210> 6																	
<211> 367																	
<212> PRT																	
<213> 大肠杆菌(Escherichia coli)																	
<220>																	
<223> 大肠杆菌甘油脱氢酶(Ec_gldA)氨基酸序列																	
<400> 6																	
Met	Asp	Arg	Ile	Ile	Gln	Ser	Pro	Gly	Lys	Tyr	Ile	Gln	Gly	Ala	Asp		
			5						10						15		
Val	Ile	Asn	Arg	Leu	Gly	Glu	Tyr	Leu	Lys	Pro	Leu	Ala	Glu	Arg	Trp		
			20						25						30		
Leu	Val	Val	Gly	Asp	Lys	Phe	Val	Leu	Gly	Phe	Ala	Gln	Ser	Thr	Val		
			35						40						45		
Glu	Lys	Ser	Phe	Lys	Asp	Ala	Gly	Leu	Val	Val	Glu	Ile	Ala	Pro	Phe		
			50						55						60		
Gly	Gly	Glu	Cys	Ser	Gln	Asn	Glu	Ile	Asp	Arg	Leu	Arg	Gly	Ile	Ala		
					70										80		
Glu	Thr	Ala	Gln	Cys	Gly	Ala	Ile	Leu	Gly	Ile	Gly	Gly	Gly	Lys	Thr		
					85										95		
Leu	Asp	Thr	Ala	Lys	Ala	Leu	Ala	His	Phe	Met	Gly	Val	Pro	Val	Ala		

100	105	110
Ile Ala Pro Thr Ile Ala Ser Thr Asp Ala Pro Cys Ser Ala Leu Ser		
115	120	125
Val Ile Tyr Thr Asp Glu Gly Glu Phe Asp Arg Tyr Leu Leu Leu Pro		
130	135	140
Asn Asn Pro Asn Met Val Ile Val Asp Thr Lys Ile Val Ala Gly Ala		
145	150	155
Pro Ala Arg Leu Leu Ala Ala Gly Ile Gly Asp Ala Leu Ala Thr Trp		
165	170	175
Phe Glu Ala Arg Ala Cys Ser Arg Ser Gly Ala Thr Thr Met Ala Gly		
180	185	190
Gly Lys Cys Thr Gln Ala Ala Leu Ala Leu Ala Glu Leu Cys Tyr Asn		
195	200	205
Thr Leu Leu Glu Glu Gly Glu Lys Ala Met Leu Ala Ala Glu Gln His		
210	215	220
Val Val Thr Pro Ala Leu Glu Arg Val Ile Glu Ala Asn Thr Tyr Leu		
225	230	235
Ser Gly Val Gly Phe Glu Ser Gly Gly Leu Ala Ala Ala His Ala Val		
245	250	255
His Asn Gly Leu Thr Ala Ile Pro Asp Ala His His Tyr Tyr His Gly		
260	265	270
Glu Lys Val Ala Phe Gly Thr Leu Thr Gln Leu Val Leu Glu Asn Ala		
275	280	285
Pro Val Glu Glu Ile Glu Thr Val Ala Ala Leu Ser His Ala Val Gly		
290	295	300
Leu Pro Ile Thr Leu Ala Gln Leu Asp Ile Lys Glu Asp Val Pro Ala		
305	310	315
Lys Met Arg Ile Val Ala Glu Ala Ala Cys Ala Glu Gly Glu Thr Ile		
325	330	335
His Asn Met Pro Gly Gly Ala Thr Pro Asp Gln Val Tyr Ala Ala Leu		
340	345	350
Leu Val Ala Asp Gln Tyr Gly Gln Arg Phe Leu Gln Glu Trp Glu		
355	360	365

<210> 7

<211> 365

<212> PRT

<213> 肺炎克雷伯氏菌 (*Klebsiella pneumoniae*)

<220>

<223> 肺炎克雷伯氏菌甘油脱氢酶 (Kpne_gldA) 氨基酸

序列

<400> 7

```

Met Leu Lys Val Ile Gln Ser Pro Ala Lys Tyr Leu Gln Gly Pro Asp
1           5           10           15
Ala Ala Val Leu Phe Gly Gln Tyr Ala Lys Asn Leu Ala Glu Ser Phe
           20           25           30
Phe Val Ile Ala Asp Asp Phe Val Met Lys Leu Ala Gly Glu Lys Val
           35           40           45
Val Asn Gly Leu Gln Ser His Asp Ile Arg Cys His Ala Glu Arg Phe
           50           55           60
Asn Gly Glu Cys Ser His Ala Glu Ile Asn Arg Leu Met Ala Ile Leu
65           70           75           80
Gln Lys Gln Gly Cys Arg Gly Val Val Gly Ile Gly Gly Gly Lys Thr
           85           90           95
Leu Asp Thr Ala Lys Ala Ile Gly Tyr Tyr Gln Lys Leu Pro Val Val
           100          105          110
Val Ile Pro Thr Ile Ala Ser Thr Asp Ala Pro Thr Ser Ala Leu Ser
           115          120          125
Val Ile Tyr Thr Glu Ala Gly Glu Phe Glu Glu Tyr Leu Ile Tyr Pro
           130          135          140
Lys Asn Pro Asp Met Val Val Met Asp Thr Ala Ile Ile Ala Lys Ala
145          150          155          160
Pro Val Arg Leu Leu Val Ser Gly Met Gly Asp Ala Leu Ser Thr Trp
           165          170          175
Phe Glu Ala Lys Ala Cys Tyr Asp Ala Arg Ala Thr Ser Met Ala Gly
           180          185          190
Gly Gln Ser Thr Glu Ala Ala Leu Ser Leu Ala Arg Leu Cys Tyr Asp
           195          200          205
Thr Leu Leu Ala Glu Gly Glu Lys Ala Arg Leu Ala Ala Gln Ala Gly
           210          215          220
Val Val Thr Glu Ala Leu Glu Arg Ile Ile Glu Ala Asn Thr Tyr Leu
225          230          235          240
Ser Gly Ile Gly Phe Glu Ser Ser Gly Leu Ala Ala Ala His Ala Ile
           245          250          255
His Asn Gly Phe Thr Ile Leu Glu Glu Cys His His Leu Tyr His Gly
           260          265          270
Glu Lys Val Ala Phe Gly Thr Leu Ala Gln Leu Val Leu Gln Asn Ser
           275          280          285
Pro Met Asp Glu Ile Glu Thr Val Leu Gly Phe Cys Gln Arg Val Gly

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290	295	300
Leu Pro Val Thr Leu Ala Gln Met Gly Val Lys Glu Gly Ile Asp Ala		
305	310	315
Lys Ile Ala Ala Val Ala Lys Ala Thr Cys Ala Glu Gly Glu Thr Ile		
325	330	335
His Asn Met Pro Phe Ala Val Thr Pro Glu Ser Val His Ala Ala Ile		
340	345	350
Leu Thr Ala Asp Leu Leu Gly Gln Gln Trp Leu Ala Arg		
355	360	365
<210> 8		
<211> 367		
<212> PRT		
<213> 产气肠杆菌(Enterococcus aerogenes)		
<220>		
<223> 产气肠杆菌甘油脱氢酶(Eaer_gldA)氨基酸 序列		
<400> 8		
Met Asp Arg Ile Ile Gln Ser Pro Gly Lys Tyr Ile Gln Gly Ala Gly		
1	5	10
Ala Ile Lys Arg Leu Gly Glu Tyr Leu Lys Pro Leu Ala Glu Arg Trp		
20	25	30
Leu Ile Ile Gly Asp Lys Phe Val Leu Gly Phe Ala Glu Glu Gln Leu		
35	40	45
Arg Thr Ser Leu Gly Gly Ala Gly Leu Val Ala Glu Ile Ala Pro Phe		
50	55	60
Gly Gly Glu Cys Ser Gln Asn Glu Ile Asn Arg Leu Arg Asp Ile Ala		
65	70	75
Ser Ser Ala Gln Cys His Ala Val Leu Gly Ile Gly Gly Gly Lys Thr		
85	90	95
Leu Asp Thr Ala Lys Ala Leu Ala His Tyr Met His Leu Pro Val Val		
100	105	110
Val Ala Pro Thr Ile Ala Ser Thr Asp Ala Pro Cys Ser Ala Leu Ser		
115	120	125
Val Ile Tyr Thr Asp Asp Gly Glu Phe Glu Ser Tyr Leu Met Leu Pro		
130	135	140
His Asn Pro Asn Met Val Val Val Asp Thr Gln Ile Val Ala Ala Ala		
145	150	155
Pro Ala Arg Leu Leu Ala Ala Gly Ile Gly Asp Ala Leu Ala Thr Trp		
165	170	175

Phe Glu Ala Arg Ala Cys Ser Arg Ser Gly Ala Thr Thr Met Ala Gly
 180 185 190
 Gly Lys Cys Thr Gln Ala Ala Leu Ala Leu Ala Glu Leu Cys Tyr Asn
 195 200 205
 Thr Leu Val Glu Glu Gly Glu Lys Ala Met Leu Ala Ala Glu Gln His
 210 215 220
 Val Val Thr Pro Ala Leu Glu Arg Val Ile Glu Ala Asn Thr Tyr Leu
 225 230 235 240
 Ser Gly Val Gly Phe Glu Ser Gly Gly Leu Ala Ala Ala His Ala Ile
 245 250 255
 His Asn Gly Leu Thr Ala Ile Pro Asp Ala His His Phe Tyr His Gly
 260 265 270
 Glu Lys Val Ala Phe Gly Thr Leu Thr Gln Leu Val Leu Glu Asn Ala
 275 280 285
 Pro Val Glu Glu Ile Glu Thr Ala Ala Ala Leu Cys His Ser Val Gly
 290 295 300
 Leu Pro Ile Thr Leu Ala Gln Leu Asp Ile Lys Gly Asp Ile Pro Ala
 305 310 315 320
 Lys Met Arg Thr Val Ala Glu Ala Ala Cys Ala Glu Gly Glu Thr Ile
 325 330 335
 His Asn Met Pro Gly Gly Ala Cys Ala Asp Gln Val Tyr Ala Ala Leu
 340 345 350
 Leu Val Ala Asp Gln Tyr Gly Gln Arg Phe Leu Gln Glu Trp Glu
 355 360 365

<210> 9

<211> 364

<212> PRT

<213> 阿氏耶尔森氏菌(*Yersinia aldovae*)

<220>

<223> 阿氏耶尔森氏菌甘油脱氢酶(Eaer_gldA)氨基酸序列

<400> 9

Met Leu Lys Val Ile Gln Ser Pro Ser Lys Tyr Ile Gln Gly Ala Asn
 1 5 10 15
 Ala Leu Gln Ser Ile Gly Glu Phe Ala Lys Leu Leu Ala Asn Asn Tyr
 20 25 30
 Phe Ile Ile Ala Asp Asp Phe Val Met Lys Leu Thr Ala Asp Thr Val
 35 40 45
 Gly Thr Ser Leu Gln Thr Cys Glu Leu Lys Ser His Phe Ser Arg Phe
 50 55 60

Asn Gly Glu Cys Ser Arg Gln Glu Ile Glu Arg Leu Thr Val Glu Leu			
65	70	75	80
Lys Lys Tyr Gly Cys Asn Gly Val Ile Gly Ile Gly Gly Gly Lys Thr			
	85	90	95
Leu Asp Thr Ala Lys Ala Ile Ala His Tyr Gln His Ile Pro Val Val			
	100	105	110
Val Val Pro Thr Ile Ala Ser Thr Asp Ala Pro Thr Ser Ala Leu Ser			
	115	120	125
Val Ile Tyr Thr Glu Gln Gly Glu Phe Ala Glu Tyr Leu Ile Tyr Pro			
	130	135	140
Lys Asn Pro Asp Ile Val Leu Met Asp Thr Thr Ile Ile Ala Lys Ala			
145	150	155	160
Pro Val Arg Leu Leu Val Ala Gly Met Gly Asp Ala Leu Ser Thr Tyr			
	165	170	175
Phe Glu Ala Gln Ala Cys Phe Asp Ala Lys Ala Ile Ser Met Ala Gly			
	180	185	190
Gly Ala Ser Thr Leu Ala Ala Ile Thr Leu Ala Arg Leu Cys Tyr Glu			
	195	200	205
Thr Leu Leu Ala Glu Gly Tyr Lys Ala Lys Leu Ala Val Glu Ala Gly			
	210	215	220
Val Val Thr Glu Ala Val Glu Arg Ile Ile Glu Ala Asn Thr Tyr Leu			
225	230	235	240
Ser Gly Ile Gly Phe Glu Ser Ser Gly Leu Ala Ala Ala His Ala Ile			
	245	250	255
His Asn Gly Phe Thr Val Leu Glu Glu Cys His His Leu Tyr His Gly			
	260	265	270
Glu Lys Val Ala Phe Gly Thr Leu Thr Gln Leu Val Leu Gln Asn Ser			
	275	280	285
Ser Met Glu Glu Ile Glu Thr Val Leu Ser Phe Cys Gln Gln Leu Gly			
	290	295	300
Leu Pro Ile Thr Leu Ala Glu Met Gly Val Thr Gln Asp Leu Glu Cys			
305	310	315	320
Lys Ile Arg Ala Val Ala Gln Ala Ser Cys Ala Glu Gly Glu Thr Ile			
	325	330	335
His Asn Met Pro Phe Lys Val Thr Ala Asp Ser Val Tyr Ala Ala Ile			
	340	345	350
Ile Val Ala Asp Arg Leu Gly Gln Ala Phe Leu Asn			
	355	360	

<210> 10

<211> 584

<212> PRT

<213> 酿酒酵母(*Saccharomyces cerevisiae*)

<220>

<223> 酿酒酵母二羟基丙酮激酶(酿酒酵母DAK1)氨基酸
序列

<400> 10

Met	Ser	Ala	Lys	Ser	Phe	Glu	Val	Thr	Asp	Pro	Val	Asn	Ser	Ser	Leu
1				5					10					15	
Lys	Gly	Phe	Ala	Leu	Ala	Asn	Pro	Ser	Ile	Thr	Leu	Val	Pro	Glu	Glu
			20					25					30		
Lys	Ile	Leu	Phe	Arg	Lys	Thr	Asp	Ser	Asp	Lys	Ile	Ala	Leu	Ile	Ser
		35				40					45				
Gly	Gly	Gly	Ser	Gly	His	Glu	Pro	Thr	His	Ala	Gly	Phe	Ile	Gly	Lys
	50					55				60					
Gly	Met	Leu	Ser	Gly	Ala	Val	Val	Gly	Glu	Ile	Phe	Ala	Ser	Pro	Ser
65					70				75					80	
Thr	Lys	Gln	Ile	Leu	Asn	Ala	Ile	Arg	Leu	Val	Asn	Glu	Asn	Ala	Ser
				85				90						95	
Gly	Val	Leu	Leu	Ile	Val	Lys	Asn	Tyr	Thr	Gly	Asp	Val	Leu	His	Phe
		100						105					110		
Gly	Leu	Ser	Ala	Glu	Arg	Ala	Arg	Ala	Leu	Gly	Ile	Asn	Cys	Arg	Val
		115					120						125		
Ala	Val	Ile	Gly	Asp	Asp	Val	Ala	Val	Gly	Arg	Glu	Lys	Gly	Gly	Met
		130					135						140		
Val	Gly	Arg	Arg	Ala	Leu	Ala	Gly	Thr	Val	Leu	Val	His	Lys	Ile	Val
145					150					155				160	
Gly	Ala	Phe	Ala	Glu	Glu	Tyr	Ser	Ser	Lys	Tyr	Gly	Leu	Asp	Gly	Thr
				165					170					175	
Ala	Lys	Val	Ala	Lys	Ile	Ile	Asn	Asp	Asn	Leu	Val	Thr	Ile	Gly	Ser
				180				185						190	
Ser	Leu	Asp	His	Cys	Lys	Val	Pro	Gly	Arg	Lys	Phe	Glu	Ser	Glu	Leu
		195						200						205	
Asn	Glu	Lys	Gln	Met	Glu	Leu	Gly	Met	Gly	Ile	His	Asn	Glu	Pro	Gly
		210					215							220	
Val	Lys	Val	Leu	Asp	Pro	Ile	Pro	Ser	Thr	Glu	Asp	Leu	Ile	Ser	Lys
225					230					235				240	
Tyr	Met	Leu	Pro	Lys	Leu	Leu	Asp	Pro	Asn	Asp	Lys	Asp	Arg	Ala	Phe
				245						250				255	

Val	Lys	Phe	Asp	Glu	Asp	Asp	Glu	Val	Val	Leu	Leu	Val	Asn	Asn	Leu
260				265				270							
Gly	Gly	Val	Ser	Asn	Phe	Val	Ile	Ser	Ser	Ile	Thr	Ser	Lys	Thr	Thr
275				280				285							
Asp	Phe	Leu	Lys	Glu	Asn	Tyr	Asn	Ile	Thr	Pro	Val	Gln	Thr	Ile	Ala
290				295				300							
Gly	Thr	Leu	Met	Thr	Ser	Phe	Asn	Gly	Asn	Gly	Phe	Ser	Ile	Thr	Leu
305				310				315				320			
Leu	Asn	Ala	Thr	Lys	Ala	Thr	Lys	Ala	Leu	Gln	Ser	Asp	Phe	Glu	Glu
325				330				335							
Ile	Lys	Ser	Val	Leu	Asp	Leu	Leu	Asn	Ala	Phe	Thr	Asn	Ala	Pro	Gly
340				345				350							
Trp	Pro	Ile	Ala	Asp	Phe	Glu	Lys	Thr	Ser	Ala	Pro	Ser	Val	Asn	Asp
355				360				365							
Asp	Leu	Leu	His	Asn	Glu	Val	Thr	Ala	Lys	Ala	Val	Gly	Thr	Tyr	Asp
370				375				380							
Phe	Asp	Lys	Phe	Ala	Glu	Trp	Met	Lys	Ser	Gly	Ala	Glu	Gln	Val	Ile
385				390				395				400			
Lys	Ser	Glu	Pro	His	Ile	Thr	Glu	Leu	Asp	Asn	Gln	Val	Gly	Asp	Gly
405				410				415							
Asp	Cys	Gly	Tyr	Thr	Leu	Val	Ala	Gly	Val	Lys	Gly	Ile	Thr	Glu	Asn
420				425				430							
Leu	Asp	Lys	Leu	Ser	Lys	Asp	Ser	Leu	Ser	Gln	Ala	Val	Ala	Gln	Ile
435				440				445							
Ser	Asp	Phe	Ile	Glu	Gly	Ser	Met	Gly	Gly	Thr	Ser	Gly	Gly	Leu	Tyr
450				455				460							
Ser	Ile	Leu	Leu	Ser	Gly	Phe	Ser	His	Gly	Leu	Ile	Gln	Val	Cys	Lys
465				470				475				480			
Ser	Lys	Asp	Glu	Pro	Val	Thr	Lys	Glu	Ile	Val	Ala	Lys	Ser	Leu	Gly
485				490				495							
Ile	Ala	Leu	Asp	Thr	Leu	Tyr	Lys	Tyr	Thr	Lys	Ala	Arg	Lys	Gly	Ser
500				505				510							
Ser	Thr	Met	Ile	Asp	Ala	Leu	Glu	Pro	Phe	Val	Lys	Glu	Phe	Thr	Ala
515				520				525							
Ser	Lys	Asp	Phe	Asn	Lys	Ala	Val	Lys	Ala	Ala	Glu	Glu	Gly	Ala	Lys
530				535				540							
Ser	Thr	Ala	Thr	Phe	Glu	Ala	Lys	Phe	Gly	Arg	Ala	Ser	Tyr	Val	Gly
545				550				555				560			
Asp	Ser	Ser	Gln	Val	Glu	Asp	Pro	Gly	Ala	Val	Gly	Leu	Cys	Glu	Phe

565	570	575
Leu Lys Gly Val Gln Ser Ala Leu		
580		
<210> 11		
<211> 572		
<212> PRT		
<213> 肺炎克雷伯氏菌 (<i>Klebsiella pneumoniae</i>)		
<220>		
<223> 肺炎克雷伯氏菌二羟基丙酮激酶 (Kpne_dhaK) 氨基酸		
序列		
<400> 11		
Met Thr Thr Lys Gln Phe Gln Phe Asp Ser Asp Pro Leu Asn Ser Ala		
1 5 10 15		
Leu Ala Ala Thr Ala Glu Ala Ser Gly Leu Ala Tyr Leu Pro Lys Ser		
20 25 30		
Lys Val Ile Tyr Tyr Pro Leu Thr Asn Asp Lys Val Thr Leu Ile Ser		
35 40 45		
Gly Gly Gly Ala Gly His Glu Pro Ala Gln Thr Gly Phe Val Gly Pro		
50 55 60		
Gly Leu Leu Asp Ala Ala Val Ser Gly Gln Ile Phe Ala Ser Pro Ser		
65 70 75 80		
Thr Lys Gln Ile Ile Ala Gly Val Asn Ala Val Lys Ser Gln Arg Gly		
85 90 95		
Ser Ile Ile Ile Val Met Asn Tyr Thr Gly Asp Val Ile His Phe Gly		
100 105 110		
Met Ala Ala Glu Gln Leu Arg Ser Arg Tyr Asp Tyr His Ala Glu Leu		
115 120 125		
Val Ser Ile Gly Asp Asp Ile Ser Val Asn Lys Lys Ala Gly Arg Arg		
130 135 140		
Gly Leu Ala Gly Thr Val Leu Val His Lys Ile Ala Gly His Leu Ala		
145 150 155 160		
Arg Asp Gly Trp Asp Val Gly Val Leu Ala Glu Ala Leu Arg Thr Thr		
165 170 175		
Ala Ala Asn Leu Ala Thr Val Ala Ala Ser Leu Glu His Cys Thr Val		
180 185 190		
Pro Gly Arg Lys Phe Glu Thr Glu Leu Ala Ala Asp Glu Met Glu Ile		
195 200 205		
Gly Met Gly Ile His Asn Glu Pro Gly Val Lys Thr Ile Lys Ile Gly		
210 215 220		

Lys Val Glu Ser Leu Leu Asp Glu Leu Val Asp Lys Phe Glu Pro Ser			
225	230	235	240
Lys Gln Asp Phe Val Pro Phe Asn Lys Gly Asp Glu Val Val Leu Leu			
	245	250	255
Val Asn Ser Leu Gly Gly Val Ser Ser Leu Glu Leu His Ala Ile Ala			
	260	265	270
Asn Ile Ala Gln Thr Lys Phe Glu Lys Val Leu Gly Val Lys Thr Val			
	275	280	285
Arg Leu Ile Val Gly Asn Phe Met Ala Ala Phe Asn Gly Pro Gly Phe			
	290	295	300
Ser Leu Thr Leu Leu Asn Val Thr Thr Thr Ala Lys Lys Gly Asn Phe			
305	310	315	320
Asp Val Leu Gly Ala Leu Asp Ala Pro Val Ser Thr Ala Ala Trp Pro			
	325	330	335
Ser Leu Gln Gln Lys Asp Lys Pro Ala Asn Gly Gly Val Gln Glu Glu			
	340	345	350
Lys Glu Thr Asp Ser Asp Lys Pro Ala Glu Pro Thr Gly Ile Lys Ala			
	355	360	365
Asp Gly Lys Leu Phe Lys Ala Met Ile Glu Ser Ala Val Asp Asp Leu			
	370	375	380
Lys Lys Glu Glu Pro Gln Ile Thr Lys Tyr Asp Thr Ile Ala Gly Asp			
385	390	395	400
Gly Asp Cys Gly Glu Thr Leu Leu Ala Gly Gly Asp Gly Ile Leu Asp			
	405	410	415
Ala Ile Lys Asn Lys Lys Ile Asp Leu Asp Asp Ala Ala Gly Val Ala			
	420	425	430
Asp Ile Ser His Ile Val Glu Asn Ser Met Gly Gly Thr Ser Gly Gly			
	435	440	445
Leu Tyr Ser Ile Phe Phe Ser Gly Leu Val Val Gly Ile Lys Glu Thr			
	450	455	460
Lys Ala Lys Glu Leu Ser Val Asp Val Phe Ala Lys Ala Cys Glu Thr			
465	470	475	480
Ala Leu Glu Thr Leu Ser Lys Tyr Thr Gln Ala Arg Val Gly Asp Arg			
	485	490	495
Thr Leu Met Asp Ala Leu Val Pro Phe Val Glu Thr Leu Ser Lys Thr			
	500	505	510
Lys Asp Phe Ala Lys Ala Val Glu Ala Ala Arg Lys Gly Ala Asp Glu			
	515	520	525
Thr Ser Lys Leu Pro Ala Asn Phe Gly Arg Ala Ser Tyr Val Asn Glu			

530	535	540
Glu Gly Leu Glu Asn Ile Pro Asp Pro Gly Ala Leu Gly Leu Ala Val		
545	550	555
Ile Phe Glu Gly Leu Leu Lys Ala Trp Glu Lys Lys		560
565	570	
<210> 12		
<211> 580		
<212> PRT		
<213> 解脂耶氏酵母 (Yarrowia lipolytica)		
<220>		
<223> 解脂耶氏酵母二羟基丙酮激酶 (Ylip_DAK1) 氨基酸		
序列		
<400> 12		
Met Asp Lys His Phe Ile Asn Asp Pro Glu Val Leu Val Leu Asp Gly		
1 5 10 15		
Leu Lys Ser Leu Ala Asp Met Asn Lys Thr Leu Thr Val His Glu Glu		
20 25 30		
Gly Lys Phe Ile Tyr Phe His Asp Tyr Asn Lys Lys Asn Val Ser Val		
35 40 45		
Ile Ser Gly Gly Gly Ala Gly His Glu Pro Thr His Ser Ser Phe Val		
50 55 60		
Gly Lys Gly Met Leu Thr Ala Ala Val Ser Gly Ser Ile Phe Ala Ser		
65 70 75 80		
Pro Ser Ser Lys Gln Ile Tyr Thr Gly Ile Lys Gln Val Glu Ser Glu		
85 90 95		
Ala Gly Thr Leu Val Ile Cys Lys Asn Tyr Thr Gly Asp Ile Leu His		
100 105 110		
Phe Gly Met Ala Leu Glu Lys Gln Arg Thr Ala Gly Lys Lys Ala Glu		
115 120 125		
Leu Ile Ala Val Ala Asp Asp Val Ser Val Gly Arg Lys Lys Ser Gly		
130 135 140		
Lys Val Gly Arg Arg Gly Leu Ser Gly Thr Val Leu Val His Lys Ile		
145 150 155 160		
Ala Gly Ala Ala Ala Ala Arg Gly Leu Pro Leu Glu Ala Val Thr Thr		
165 170 175		
Ile Ala Lys Ala Ala Ile Asp Asn Leu Val Ser Ile Gly Ala Ser Leu		
180 185 190		
Ala His Val His Val Pro Gly His Glu Pro Ile Ala Lys Glu Asp Glu		
195 200 205		

Met Lys His Asp Glu Met Glu Leu Gly Met Gly Ile His Asn Glu Pro		
210	215	220
Gly Cys Lys Arg Ile Ser Pro Ile Pro Ser Ile Asp Asp Leu Ile Ala		
225	230	235 240
Gln Met Leu Lys Gln Met Leu Asp Gln Ser Asp Lys Asp Arg Ala Tyr		
245	250	255
Val Lys Ile Glu Gly Asp Asp Glu Val Val Leu Leu Met Asn Asn Leu		
260	265	270
Gly Gly Leu Ser Met Leu Glu Phe Ser Ala Ile Ser His Lys Val Lys		
275	280	285
Glu Ala Leu Ala Lys Glu Tyr Lys Ile Asn Pro Val Arg Ile Phe Ala		
290	295	300
Gly Pro Phe Thr Thr Ser Leu Asn Gly Leu Gly Phe Gly Ile Thr Leu		
305	310	315 320
Leu Arg Thr Thr Asp Arg Val Lys Val Glu Gly Glu Glu Tyr Ser Leu		
325	330	335
Val Asp Leu Ile Asp Gln Pro Val Glu Ala Ile Gly Trp Pro Leu Cys		
340	345	350
Gln Pro Ser Asp Leu Lys Ser Lys Asn Lys Ile Gly Asn Val Ser Ile		
355	360	365
Glu Glu Gly Gln Lys Asp Val Lys Ser Pro Val Thr Val Asp Lys Glu		
370	375	380
Lys Val Arg Gln Ala Ile Val Asn Ser Met Glu Asn Leu Ile Lys Ala		
385	390	395 400
Glu Pro Lys Ile Thr Lys Phe Asp Thr Met Ala Gly Asp Gly Asp Cys		
405	410	415
Gly Thr Thr Leu Lys Arg Gly Ala Glu Gly Val Leu Lys Phe Val Lys		
420	425	430
Ser Asp Lys Phe Ser Asp Asp Pro Ile Arg Ile Val Arg Asp Ile Ala		
435	440	445
Asp Val Ile Glu Asp Asn Met Asp Gly Thr Ser Gly Ala Leu Tyr Ala		
450	455	460
Ile Phe Phe His Gly Phe Ala Lys Gly Met Lys Asp Thr Leu Glu Lys		
465	470	475 480
Ser Lys Asp Ile Ser Ser Lys Thr Trp Ala Ala Gly Leu Lys Val Ala		
485	490	495
Leu Asp Thr Leu Phe Lys Tyr Thr Pro Ala Arg Pro Gly Asp Ser Thr		
500	505	510
Met Cys Asp Ala Leu Val Pro Phe Val Glu Thr Phe Val Lys Thr Asn		

515	520	525
Asp Leu Asn Ala Ala Val Glu Glu Ala Arg Lys Gly Ala Asp Ala Thr		
530	535	540
Ala Asp Met Gln Ala Lys Leu Gly Arg Ala Val Tyr Val Gly Asp Asp		
545	550	555
Val Lys Val Pro Asp Ala Gly Ala Leu Gly Val Val Ala Ile Val Glu		
565	570	575
Gly Phe Thr Lys		
580		
<210> 13		
<211> 580		
<212> PRT		
<213> 粟酒裂殖酵母 (Schizosaccharomyces pombe)		
<220>		
<223> 粟酒裂殖酵母二羟基丙酮激酶 (Spom_DAK1) 氨基酸序列		
<400> 13		
Met Asp Lys His Phe Ile Asn Asp Pro Glu Val Leu Val Leu Asp Gly		
1 5 10 15		
Leu Lys Ser Leu Ala Asp Met Asn Lys Thr Leu Thr Val His Glu Glu		
20 25 30		
Gly Lys Phe Ile Tyr Phe His Asp Tyr Asn Lys Lys Asn Val Ser Val		
35 40 45		
Ile Ser Gly Gly Gly Ala Gly His Glu Pro Thr His Ser Ser Phe Val		
50 55 60		
Gly Lys Gly Met Leu Thr Ala Ala Val Ser Gly Ser Ile Phe Ala Ser		
65 70 75 80		
Pro Ser Ser Lys Gln Ile Tyr Thr Gly Ile Lys Gln Val Glu Ser Glu		
85 90 95		
Ala Gly Thr Leu Val Ile Cys Lys Asn Tyr Thr Gly Asp Ile Leu His		
100 105 110		
Phe Gly Met Ala Leu Glu Lys Gln Arg Thr Ala Gly Lys Lys Ala Glu		
115 120 125		
Leu Ile Ala Val Ala Asp Asp Val Ser Val Gly Arg Lys Lys Ser Gly		
130 135 140		
Lys Val Gly Arg Arg Gly Leu Ser Gly Thr Val Leu Val His Lys Ile		
145 150 155 160		
Ala Gly Ala Ala Ala Ala Arg Gly Leu Pro Leu Glu Ala Val Thr Thr		
165 170 175		
Ile Ala Lys Ala Ala Ile Asp Asn Leu Val Ser Ile Gly Ala Ser Leu		

180	185	190
Ala His Val His Val Pro Gly	His Glu Pro Ile Ala Lys Glu Asp Glu	
195	200	205
Met Lys His Asp Glu Met Glu Leu Gly Met Gly Ile His Asn Glu Pro		
210	215	220
Gly Cys Lys Arg Ile Ser Pro Ile Pro Ser Ile Asp Asp Leu Ile Ala		
225	230	235
Gln Met Leu Lys Gln Met Leu Asp Gln Ser Asp Lys Asp Arg Ala Tyr		
245	250	255
Val Lys Ile Glu Gly Asp Asp Glu Val Val Leu Leu Met Asn Asn Leu		
260	265	270
Gly Gly Leu Ser Met Leu Glu Phe Ser Ala Ile Ser His Lys Val Lys		
275	280	285
Glu Ala Leu Ala Lys Glu Tyr Lys Ile Asn Pro Val Arg Ile Phe Ala		
290	295	300
Gly Pro Phe Thr Thr Ser Leu Asn Gly Leu Gly Phe Gly Ile Thr Leu		
305	310	315
Leu Arg Thr Thr Asp Arg Val Lys Val Glu Gly Glu Glu Tyr Ser Leu		
325	330	335
Val Asp Leu Ile Asp Gln Pro Val Glu Ala Ile Gly Trp Pro Leu Cys		
340	345	350
Gln Pro Ser Asp Leu Lys Ser Lys Asn Lys Ile Gly Asn Val Ser Ile		
355	360	365
Glu Glu Gly Gln Lys Asp Val Lys Ser Pro Val Thr Val Asp Lys Glu		
370	375	380
Lys Val Arg Gln Ala Ile Val Asn Ser Met Glu Asn Leu Ile Lys Ala		
385	390	395
Glu Pro Lys Ile Thr Lys Phe Asp Thr Met Ala Gly Asp Gly Asp Cys		
405	410	415
Gly Thr Thr Leu Lys Arg Gly Ala Glu Gly Val Leu Lys Phe Val Lys		
420	425	430
Ser Asp Lys Phe Ser Asp Asp Pro Ile Arg Ile Val Arg Asp Ile Ala		
435	440	445
Asp Val Ile Glu Asp Asn Met Asp Gly Thr Ser Gly Ala Leu Tyr Ala		
450	455	460
Ile Phe Phe His Gly Phe Ala Lys Gly Met Lys Asp Thr Leu Glu Lys		
465	470	475
Ser Lys Asp Ile Ser Ser Lys Thr Trp Ala Ala Gly Leu Lys Val Ala		
485	490	495

Leu Asp Thr Leu Phe Lys Tyr Thr Pro Ala Arg Pro Gly Asp Ser Thr
 500 505 510
 Met Cys Asp Ala Leu Val Pro Phe Val Glu Thr Phe Val Lys Thr Asn
 515 520 525
 Asp Leu Asn Ala Ala Val Glu Glu Ala Arg Lys Gly Ala Asp Ala Thr
 530 535 540
 Ala Asp Met Gln Ala Lys Leu Gly Arg Ala Val Tyr Val Gly Asp Asp
 545 550 555 560
 Val Lys Val Pro Asp Ala Gly Ala Leu Gly Val Val Ala Ile Val Glu
 565 570 575
 Gly Phe Thr Lys
 580

<210> 14

<211> 291

<212> PRT

<213> 斑马鱼 (Danio rerio)

<220>

<223> 斑马鱼水通道蛋白9 (Drer_T3) 氨基酸序列

<400> 14

Met Glu Tyr Leu Glu Asn Ile Arg Asn Leu Arg Gly Arg Cys Val Leu
 1 5 10 15
 Arg Arg Asp Ile Ile Arg Glu Phe Leu Ala Glu Leu Leu Gly Thr Phe
 20 25 30
 Val Leu Ile Leu Phe Gly Cys Gly Ser Val Ala Gln Thr Val Leu Ser
 35 40 45
 Arg Glu Ala Lys Gly Gln Leu Leu Thr Ile His Phe Gly Phe Thr Leu
 50 55 60
 Gly Val Met Leu Ala Val Tyr Met Ala Gly Gly Val Ser Gly Gly His
 65 70 75 80
 Val Asn Pro Ala Val Ser Leu Ala Met Val Val Leu Arg Lys Leu Pro
 85 90 95
 Leu Lys Lys Phe Pro Val Tyr Val Leu Ala Gln Phe Leu Gly Ala Phe
 100 105 110
 Phe Gly Ser Cys Ala Val Tyr Cys Leu Tyr Tyr Asp Ala Phe Thr Glu
 115 120 125
 Phe Ala Asn Gly Glu Leu Ala Val Thr Gly Pro Asn Val Thr Ala Gly
 130 135 140
 Ile Phe Ala Ser Tyr Pro Arg Glu Gly Leu Ser Leu Leu Asn Gly Phe
 145 150 155 160

Ile Asp Gln Val Ile Gly Ala Gly Ala Leu Val Leu Cys Ile Leu Ala
 165 170 175
 Val Val Asp Lys Lys Asn Ile Gly Ala Pro Lys Gly Met Glu Pro Leu
 180 185 190
 Leu Val Gly Leu Ser Ile Leu Ala Ile Gly Val Ser Met Ala Leu Asn
 195 200 205
 Cys Gly Tyr Pro Ile Asn Pro Ala Arg Asp Leu Gly Pro Arg Leu Phe
 210 215 220
 Thr Ala Ile Ala Gly Trp Gly Leu Thr Val Phe Ser Ala Gly Asn Gly
 225 230 235 240
 Trp Trp Trp Val Pro Val Val Gly Pro Met Val Gly Gly Val Val Gly
 245 250 255
 Ala Ala Ile Tyr Phe Leu Met Ile Glu Met His His Pro Glu Asn Asp
 260 265 270
 Lys Asn Leu Glu Asp Asp Asn Ser Leu Lys Asp Lys Tyr Glu Leu Asn
 275 280 285
 Thr Val Asn
 290

<210> 15

<211> 592

<212> PRT

<213> 鲁氏接合酵母 (*Zygosaccharomyces rouxii*)

<220>

<223> 鲁氏接合酵母 ZYR00E01210p (Zrou_T5) 氨基酸序列

<400> 15

Met Gly Lys Arg Thr Gln Gly Phe Met Asp Tyr Val Phe Ser Arg Thr
 1 5 10 15
 Ser Thr Ala Gly Leu Lys Gly Ala Arg Leu Arg Tyr Thr Ala Ala Ala
 20 25 30
 Val Ala Val Ile Gly Phe Ala Leu Phe Gly Tyr Asp Gln Gly Leu Met
 35 40 45
 Ser Gly Leu Ile Thr Gly Asp Gln Phe Asn Lys Glu Phe Pro Pro Thr
 50 55 60
 Lys Ser Asn Gly Asp Asn Asp Arg Tyr Ala Ser Val Ile Gln Gly Ala
 65 70 75 80
 Val Thr Ala Cys Tyr Glu Ile Gly Cys Phe Phe Gly Ser Leu Phe Val
 85 90 95
 Leu Phe Phe Gly Asp Ala Ile Gly Arg Lys Pro Leu Ile Ile Phe Gly
 100 105 110

Ala Ile Ile Val Ile Ile Gly Thr Val Ile Ser Thr Ala Pro Phe His		
115	120	125
His Ala Trp Gly Leu Gly Gln Phe Val Val Gly Arg Val Ile Thr Gly		
130	135	140
Val Gly Thr Gly Phe Asn Thr Ser Thr Ile Pro Val Trp Gln Ser Glu		
145	150	155
Met Thr Lys Pro Asn Ile Arg Gly Ala Met Ile Asn Leu Asp Gly Ser		
165	170	175
Val Ile Ala Phe Gly Thr Met Ile Ala Tyr Trp Leu Asp Phe Gly Phe		
180	185	190
Ser Phe Ile Asn Ser Ser Val Gln Trp Arg Phe Pro Val Ser Val Gln		
195	200	205
Ile Ile Phe Ala Leu Val Leu Leu Phe Gly Ile Val Arg Met Pro Glu		
210	215	220
Ser Pro Arg Trp Leu Met Ala Lys Lys Arg Pro Ala Glu Ala Arg Tyr		
225	230	235
Val Leu Ala Cys Leu Asn Asp Leu Pro Glu Asn Asp Asp Ala Ile Leu		
245	250	255
Ala Glu Met Thr Ser Leu His Glu Ala Val Asn Arg Ser Ser Asn Gln		
260	265	270
Lys Ser Gln Met Lys Ser Leu Phe Ser Met Gly Lys Gln Gln Asn Phe		
275	280	285
Ser Arg Ala Leu Ile Ala Ser Ser Thr Gln Phe Phe Gln Gln Phe Thr		
290	295	300
Gly Cys Asn Ala Ala Ile Tyr Tyr Ser Thr Val Leu Phe Gln Thr Thr		
305	310	315
Val Gln Leu Asp Arg Leu Leu Ala Met Ile Leu Gly Gly Val Phe Ala		
325	330	335
Thr Val Tyr Thr Leu Ser Thr Leu Pro Ser Phe Tyr Leu Val Glu Lys		
340	345	350
Val Gly Arg Arg Lys Met Phe Phe Phe Gly Ala Leu Gly Gln Gly Ile		
355	360	365
Ser Phe Ile Ile Thr Phe Ala Cys Leu Val Asn Pro Thr Lys Gln Asn		
370	375	380
Ala Lys Gly Ala Ala Val Gly Leu Tyr Leu Phe Ile Ile Cys Phe Gly		
385	390	395
Leu Ala Ile Leu Glu Leu Pro Trp Ile Tyr Pro Pro Glu Ile Ala Ser		
405	410	415
Met Arg Val Arg Ala Ala Thr Asn Ala Met Ser Thr Cys Thr Asn Trp		

420					425					430						
Val	Thr	Asn	Phe	Ala	Val	Val	Met	Phe	Thr	Pro	Val	Phe	Ile	Gln	Thr	
435					440					445						
Ser	Gln	Trp	Gly	Cys	Tyr	Leu	Phe	Phe	Ala	Val	Met	Asn	Phe	Ile	Tyr	
450					455					460						
Leu	Pro	Val	Ile	Phe	Phe	Phe	Tyr	Pro	Glu	Thr	Ala	Gly	Arg	Ser	Leu	
465					470					475					480	
Glu	Glu	Ile	Asp	Ile	Ile	Phe	Ala	Lys	Ala	His	Val	Asp	Gly	Thr	Leu	
485					490					495						
Pro	Trp	Met	Val	Ala	His	Arg	Leu	Pro	Lys	Leu	Ser	Met	Thr	Glu	Val	
500					505					510						
Glu	Asp	Tyr	Ser	Gln	Ser	Leu	Gly	Leu	His	Asp	Asp	Glu	Asn	Glu	Lys	
515					520					525						
Glu	Glu	Tyr	Asp	Glu	Lys	Glu	Ala	Glu	Ala	Asn	Ala	Ala	Leu	Phe	Gln	
530					535					540						
Val	Glu	Thr	Ser	Ser	Lys	Ser	Pro	Ser	Ser	Asn	Arg	Lys	Asp	Asp	Asp	
545					550					555					560	
Ala	Pro	Ile	Glu	His	Asn	Glu	Val	Gln	Glu	Ser	Asn	Asp	Asn	Ser	Ser	
565					570					575						
Asn	Ser	Ser	Asn	Val	Glu	Ala	Pro	Ile	Pro	Val	His	His	Asn	Asp	Pro	
580					585					590						

Met	Thr	Asn	Pro	Val	Ile	Gly	Thr	Pro	Trp	Gln	Lys	Leu	Asp	Arg	Pro
1				5					10					15	
Val	Ser	Glu	Glu	Ala	Ile	Glu	Gly	Met	Asp	Lys	Tyr	Trp	Arg	Val	Ala
			20					25					30		
Asn	Tyr	Met	Ser	Ile	Gly	Gln	Ile	Tyr	Leu	Arg	Ser	Asn	Pro	Leu	Met
		35					40					45			
Lys	Glu	Pro	Phe	Thr	Arg	Asp	Asp	Val	Lys	His	Arg	Leu	Val	Gly	His
	50					55					60				
Trp	Gly	Thr	Thr	Pro	Gly	Leu	Asn	Phe	Leu	Leu	Ala	His	Ile	Asn	Arg
65					70						75				80

Leu Ile Ala Asp His Gln Gln Asn Thr Val Phe Ile Met Gly Pro Gly		
85	90	95
His Gly Gly Pro Ala Gly Thr Ala Gln Ser Tyr Ile Asp Gly Thr Tyr		
100	105	110
Thr Glu Tyr Tyr Pro Asn Ile Thr Lys Asp Glu Ala Gly Leu Gln Lys		
115	120	125
Phe Phe Arg Gln Phe Ser Tyr Pro Gly Gly Ile Pro Ser His Phe Ala		
130	135	140
Pro Glu Thr Pro Gly Ser Ile His Glu Gly Gly Glu Leu Gly Tyr Ala		
145	150	155
Leu Ser His Ala Tyr Gly Ala Ile Met Asp Asn Pro Ser Leu Phe Val		
165	170	175
Pro Cys Ile Ile Gly Asp Gly Glu Ala Glu Thr Gly Pro Leu Ala Thr		
180	185	190
Gly Trp Gln Ser Asn Lys Leu Val Asn Pro Arg Thr Asp Gly Ile Val		
195	200	205
Leu Pro Ile Leu His Leu Asn Gly Tyr Lys Ile Ala Asn Pro Thr Ile		
210	215	220
Leu Ala Arg Ile Ser Asp Glu Glu Leu His Asp Phe Phe Arg Gly Met		
225	230	235
Gly Tyr His Pro Tyr Glu Phe Val Ala Gly Phe Asp Asn Glu Asp His		
245	250	255
Leu Ser Ile His Arg Arg Phe Ala Glu Leu Phe Glu Thr Ile Phe Asp		
260	265	270
Glu Ile Cys Asp Ile Lys Ala Ala Ala Gln Thr Asp Asp Met Thr Arg		
275	280	285
Pro Phe Tyr Pro Met Leu Ile Phe Arg Thr Pro Lys Gly Trp Thr Cys		
290	295	300
Pro Lys Phe Ile Asp Gly Lys Lys Thr Glu Gly Ser Trp Arg Ala His		
305	310	315
Gln Val Pro Leu Ala Ser Ala Arg Asp Thr Glu Ala His Phe Glu Val		
325	330	335
Leu Lys Gly Trp Met Glu Ser Tyr Lys Pro Glu Glu Leu Phe Asn Ala		
340	345	350
Asp Gly Ser Ile Lys Glu Asp Val Thr Ala Phe Met Pro Lys Gly Glu		
355	360	365
Leu Arg Ile Gly Ala Asn Pro Asn Ala Asn Gly Gly Arg Ile Arg Glu		
370	375	380
Asp Leu Lys Leu Pro Glu Leu Asp Gln Tyr Glu Ile Thr Gly Val Lys		

385	390	395	400
Glu Tyr Gly His Gly Trp Gly Gln Val Glu Ala Pro Arg Ser Leu Gly			
	405	410	415
Ala Tyr Cys Arg Asp Ile Ile Lys Asn Asn Pro Asp Ser Phe Arg Val			
	420	425	430
Phe Gly Pro Asp Glu Thr Ala Ser Asn Arg Leu Asn Ala Thr Tyr Glu			
	435	440	445
Val Thr Lys Lys Gln Trp Asp Asn Gly Tyr Leu Ser Ala Leu Val Asp			
	450	455	460
Glu Asn Met Ala Val Thr Gly Gln Val Val Glu Gln Leu Ser Glu His			
465	470	475	480
Gln Cys Glu Gly Phe Leu Glu Ala Tyr Leu Leu Thr Gly Arg His Gly			
	485	490	495
Ile Trp Ser Ser Tyr Glu Ser Phe Val His Val Ile Asp Ser Met Leu			
	500	505	510
Asn Gln His Ala Lys Trp Leu Glu Ala Thr Val Arg Glu Ile Pro Trp			
	515	520	525
Arg Lys Pro Ile Ser Ser Val Asn Leu Leu Val Ser Ser His Val Trp			
	530	535	540
Arg Gln Asp His Asn Gly Phe Ser His Gln Asp Pro Gly Val Thr Ser			
545	550	555	560
Val Leu Leu Asn Lys Thr Phe Asn Asn Asp His Val Thr Asn Ile Tyr			
	565	570	575
Phe Ala Thr Asp Ala Asn Met Leu Leu Ala Ile Ala Glu Lys Cys Phe			
	580	585	590
Lys Ser Thr Asn Lys Ile Asn Ala Ile Phe Ala Gly Lys Gln Pro Ala			
	595	600	605
Ala Thr Trp Ile Thr Leu Asp Glu Val Arg Ala Glu Leu Glu Ala Gly			
	610	615	620
Ala Ala Glu Trp Lys Trp Ala Ser Asn Ala Lys Ser Asn Asp Glu Val			
625	630	635	640
Gln Val Val Leu Ala Ala Ala Gly Asp Val Pro Thr Gln Glu Ile Met			
	645	650	655
Ala Ala Ser Asp Ala Leu Asn Lys Met Gly Ile Lys Phe Lys Val Val			
	660	665	670
Asn Val Val Asp Leu Ile Lys Leu Gln Ser Ser Lys Glu Asn Asp Glu			
	675	680	685
Ala Met Ser Asp Glu Asp Phe Ala Asp Leu Phe Thr Ala Asp Lys Pro			
690	695	700	

Val	Leu	Phe	Ala	Tyr	His	Ser	Tyr	Ala	Gln	Asp	Val	Arg	Gly	Leu	Ile
705					710					715					720
Tyr	Asp	Arg	Pro	Asn	His	Asp	Asn	Phe	Thr	Val	Val	Gly	Tyr	Lys	Glu
				725					730					735	
Gln	Gly	Ser	Thr	Thr	Thr	Pro	Phe	Asp	Met	Val	Arg	Val	Asn	Asp	Met
			740					745					750		
Asp	Arg	Tyr	Ala	Leu	Gln	Ala	Lys	Ala	Leu	Glu	Leu	Ile	Asp	Ala	Asp
		755					760						765		
Lys	Tyr	Ala	Asp	Lys	Ile	Asn	Glu	Leu	Asn	Glu	Phe	Arg	Lys	Thr	Ala
	770					775						780			
Phe	Gln	Phe	Ala	Val	Asp	Asn	Gly	Tyr	Asp	Ile	Pro	Glu	Phe	Thr	Asp
785					790					795					800
Trp	Val	Tyr	Pro	Asp	Val	Lys	Val	Asp	Glu	Thr	Ser	Met	Leu	Ser	Ala
				805					810					815	
Thr	Ala	Ala	Thr	Ala	Gly	Asp	Asn	Glu							
			820					825							

<210> 17

<211> 825

<212> PRT

<213> 青春双歧杆菌(Bifidobacterium adolescentis)

<220>

<223> 青春双歧杆菌木酮糖-5P/果糖-6P磷酸转酮酶
(Bado_XFP.orf)氨基酸序列

<400> 17

Met	Thr	Ser	Pro	Val	Ile	Gly	Thr	Pro	Trp	Lys	Lys	Leu	Asn	Ala	Pro
1				5					10					15	
Val	Ser	Glu	Glu	Ala	Ile	Glu	Gly	Val	Asp	Lys	Tyr	Trp	Arg	Ala	Ala
				20				25					30		
Asn	Tyr	Leu	Ser	Ile	Gly	Gln	Ile	Tyr	Leu	Arg	Ser	Asn	Pro	Leu	Met
		35				40						45			
Lys	Glu	Pro	Phe	Thr	Arg	Glu	Asp	Val	Lys	His	Arg	Leu	Val	Gly	His
	50					55					60				
Trp	Gly	Thr	Thr	Pro	Gly	Leu	Asn	Phe	Leu	Ile	Gly	His	Ile	Asn	Arg
65					70				75					80	
Leu	Ile	Ala	Asp	His	Gln	Gln	Asn	Thr	Val	Ile	Ile	Met	Gly	Pro	Gly
				85				90					95		
His	Gly	Gly	Pro	Ala	Gly	Thr	Ala	Gln	Ser	Tyr	Leu	Asp	Gly	Thr	Tyr
			100					105					110		
Thr	Glu	Tyr	Phe	Pro	Asn	Ile	Thr	Lys	Asp	Glu	Ala	Gly	Leu	Gln	Lys

115	120	125
Phe Phe Arg Gln Phe Ser Tyr	Pro Gly Gly Ile Pro Ser His Tyr Ala	
130	135	140
Pro Glu Thr Pro Gly Ser Ile His Glu Gly Gly Glu Leu Gly Tyr Ala		
145	150	155
Leu Ser His Ala Tyr Gly Ala Val Met Asn Asn Pro Ser Leu Phe Val		
165	170	175
Pro Ala Ile Val Gly Asp Gly Glu Ala Glu Thr Gly Pro Leu Ala Thr		
180	185	190
Gly Trp Gln Ser Asn Lys Leu Ile Asn Pro Arg Thr Asp Gly Ile Val		
195	200	205
Leu Pro Ile Leu His Leu Asn Gly Tyr Lys Ile Ala Asn Pro Thr Ile		
210	215	220
Leu Ser Arg Ile Ser Asp Glu Glu Leu His Glu Phe Phe His Gly Met		
225	230	235
Gly Tyr Glu Pro Tyr Glu Phe Val Ala Gly Phe Asp Asn Glu Asp His		
245	250	255
Leu Ser Ile His Arg Arg Phe Ala Glu Leu Phe Glu Thr Val Phe Asp		
260	265	270
Glu Ile Cys Asp Ile Lys Ala Ala Ala Gln Thr Asp Asp Met Thr Arg		
275	280	285
Pro Phe Tyr Pro Met Ile Ile Phe Arg Thr Pro Lys Gly Trp Thr Cys		
290	295	300
Pro Lys Phe Ile Asp Gly Lys Lys Thr Glu Gly Ser Trp Arg Ser His		
305	310	315
Gln Val Pro Leu Ala Ser Ala Arg Asp Thr Glu Ala His Phe Glu Val		
325	330	335
Leu Lys Asn Trp Leu Glu Ser Tyr Lys Pro Glu Glu Leu Phe Asp Glu		
340	345	350
Asn Gly Ala Val Lys Pro Glu Val Thr Ala Phe Met Pro Thr Gly Glu		
355	360	365
Leu Arg Ile Gly Glu Asn Pro Asn Ala Asn Gly Gly Arg Ile Arg Glu		
370	375	380
Glu Leu Lys Leu Pro Lys Leu Glu Asp Tyr Glu Val Lys Glu Val Ala		
385	390	395
Glu Tyr Gly His Gly Trp Gly Gln Leu Glu Ala Thr Arg Arg Leu Gly		
405	410	415
Val Tyr Thr Arg Asp Ile Ile Lys Asn Asn Pro Asp Ser Phe Arg Ile		
420	425	430

Phe Gly Pro Asp Glu Thr Ala Ser Asn Arg Leu Gln Ala Ala Tyr Asp		
435	440	445
Val Thr Asn Lys Gln Trp Asp Ala Gly Tyr Leu Ser Ala Gln Val Asp		
450	455	460
Glu His Met Ala Val Thr Gly Gln Val Thr Glu Gln Leu Ser Glu His		
465	470	475
Gln Met Glu Gly Phe Leu Glu Gly Tyr Leu Leu Thr Gly Arg His Gly		
	485	490
Ile Trp Ser Ser Tyr Glu Ser Phe Val His Val Ile Asp Ser Met Leu		
	500	510
Asn Gln His Ala Lys Trp Leu Glu Ala Thr Val Arg Glu Ile Pro Trp		
	515	520
Arg Lys Pro Ile Ser Ser Met Asn Leu Leu Val Ser Ser His Val Trp		
	530	540
Arg Gln Asp His Asn Gly Phe Ser His Gln Asp Pro Gly Val Thr Ser		
545	550	555
Val Leu Leu Asn Lys Cys Phe Asn Asn Asp His Val Ile Gly Ile Tyr		
	565	570
Phe Pro Val Asp Ser Asn Met Leu Leu Ala Val Ala Glu Lys Cys Tyr		
	580	585
Lys Ser Thr Asn Lys Ile Asn Ala Ile Ile Ala Gly Lys Gln Pro Ala		
	595	600
Ala Thr Trp Leu Thr Leu Asp Glu Ala Arg Ala Glu Leu Glu Lys Gly		
	610	615
Ala Ala Glu Trp Lys Trp Ala Ser Asn Val Lys Ser Asn Asp Glu Ala		
625	630	635
Gln Ile Val Leu Ala Ala Thr Gly Asp Val Pro Thr Gln Glu Ile Met		
	645	650
Ala Ala Ala Asp Lys Leu Asp Ala Met Gly Ile Lys Phe Lys Val Val		
	660	665
Asn Val Val Asp Leu Val Lys Leu Gln Ser Ala Lys Glu Asn Asn Glu		
	675	680
Ala Leu Ser Asp Glu Glu Phe Ala Glu Leu Phe Thr Glu Asp Lys Pro		
	690	695
Val Leu Phe Ala Tyr His Ser Tyr Ala Arg Asp Val Arg Gly Leu Ile		
705	710	715
Tyr Asp Arg Pro Asn His Asp Asn Phe Asn Val His Gly Tyr Glu Glu		
	725	730
Gln Gly Ser Thr Thr Thr Pro Tyr Asp Met Val Arg Val Asn Asn Ile		

740	745	750
Asp Arg Tyr Glu Leu Gln Ala Glu Ala Leu Arg Met Ile Asp Ala Asp		
755	760	765
Lys Tyr Ala Asp Lys Ile Asn Glu Leu Glu Ala Phe Arg Gln Glu Ala		
770	775	780
Phe Gln Phe Ala Val Asp Asn Gly Tyr Asp His Pro Asp Tyr Thr Asp		
785	790	795
Trp Val Tyr Ser Gly Val Asn Thr Asn Lys Gln Gly Ala Ile Ser Ala		
805	810	815
Thr Ala Ala Thr Ala Gly Asp Asn Glu		
820	825	
<210> 18		
<211> 825		
<212> PRT		
<213> 乳酸双歧杆菌(Bifidobacterium lactis)		
<220>		
<223> 乳酸双歧杆菌木酮糖-5P/果糖-6P磷酸转酮酶(Blac_XFP.orf)		
氨基酸序列		
<400> 18		
Met Thr Asn Pro Val Ile Gly Thr Pro Trp Gln Lys Leu Asp Arg Pro		
1	5	10
Val Ser Glu Glu Ala Ile Glu Gly Met Asp Lys Tyr Trp Arg Val Ala		
20	25	30
Asn Tyr Met Ser Ile Gly Gln Ile Tyr Leu Arg Ser Asn Pro Leu Met		
35	40	45
Lys Glu Pro Phe Thr Arg Asp Asp Val Lys His Arg Leu Val Gly His		
50	55	60
Trp Gly Thr Thr Pro Gly Leu Asn Phe Leu Leu Ala His Ile Asn Arg		
65	70	75
Leu Ile Ala Asp His Gln Gln Asn Thr Val Phe Ile Met Gly Pro Gly		
85	90	95
His Gly Gly Pro Ala Gly Thr Ala Gln Ser Tyr Ile Asp Gly Thr Tyr		
100	105	110
Thr Glu Tyr Tyr Pro Asn Ile Thr Lys Asp Glu Ala Gly Leu Gln Lys		
115	120	125
Phe Phe Arg Gln Phe Ser Tyr Pro Gly Gly Ile Pro Ser His Phe Ala		
130	135	140
Pro Glu Thr Pro Gly Ser Ile His Glu Gly Gly Glu Leu Gly Tyr Ala		
145	150	155
		160

Leu Ser His Ala Tyr Gly Ala Ile Met Asp Asn Pro Ser Leu Phe Val		
	165	170 175
Pro Cys Ile Ile Gly Asp Gly Glu Ala Glu Thr Gly Pro Leu Ala Thr		
	180	185 190
Gly Trp Gln Ser Asn Lys Leu Val Asn Pro Arg Thr Asp Gly Ile Val		
	195	200 205
Leu Pro Ile Leu His Leu Asn Gly Tyr Lys Ile Ala Asn Pro Thr Ile		
	210	215 220
Leu Ala Arg Ile Ser Asp Glu Glu Leu His Asp Phe Phe Arg Gly Met		
225	230	235 240
Gly Tyr His Pro Tyr Glu Phe Val Ala Gly Phe Asp Asn Glu Asp His		
	245	250 255
Leu Ser Ile His Arg Arg Phe Ala Glu Leu Phe Glu Thr Ile Phe Asp		
	260	265 270
Glu Ile Cys Asp Ile Lys Ala Ala Ala Gln Thr Asp Asp Met Thr Arg		
	275	280 285
Pro Phe Tyr Pro Met Leu Ile Phe Arg Thr Pro Lys Gly Trp Thr Cys		
	290	295 300
Pro Lys Phe Ile Asp Gly Lys Lys Thr Glu Gly Ser Trp Arg Ala His		
305	310	315 320
Gln Val Pro Leu Ala Ser Ala Arg Asp Thr Glu Ala His Phe Glu Val		
	325	330 335
Leu Lys Gly Trp Met Glu Ser Tyr Lys Pro Glu Glu Leu Phe Asn Ala		
	340	345 350
Asp Gly Ser Ile Lys Glu Asp Val Thr Ala Phe Met Pro Lys Gly Glu		
	355	360 365
Leu Arg Ile Gly Ala Asn Pro Asn Ala Asn Gly Gly Arg Ile Arg Glu		
	370	375 380
Asp Leu Lys Leu Pro Glu Leu Asp Gln Tyr Glu Ile Thr Gly Val Lys		
385	390	395 400
Glu Tyr Gly His Gly Trp Gly Gln Val Glu Ala Pro Arg Ser Leu Gly		
	405	410 415
Ala Tyr Cys Arg Asp Ile Ile Lys Asn Asn Pro Asp Ser Phe Arg Val		
	420	425 430
Phe Gly Pro Asp Glu Thr Ala Ser Asn Arg Leu Asn Ala Thr Tyr Glu		
	435	440 445
Val Thr Lys Lys Gln Trp Asp Asn Gly Tyr Leu Ser Ala Leu Val Asp		
	450	455 460
Glu Asn Met Ala Val Thr Gly Gln Val Val Glu Gln Leu Ser Glu His		

465	470	475	480
Gln Cys Glu Gly Phe	Leu Glu Ala Tyr	Leu Leu Thr Gly	Arg His Gly
	485	490	495
Ile Trp Ser Ser Tyr	Glu Ser Phe Val	His Val Ile Asp	Ser Met Leu
	500	505	510
Asn Gln His Ala Lys	Trp Leu Glu Ala	Thr Val Arg Glu	Ile Pro Trp
	515	520	525
Arg Lys Pro Ile Ser	Ser Val Asn Leu	Leu Val Ser Ser	His Val Trp
	530	535	540
Arg Gln Asp His Asn	Gly Phe Ser His	Gln Asp Pro Gly	Val Thr Ser
545	550	555	560
Val Leu Leu Asn Lys	Thr Phe Asn Asn	Asp His Val Thr	Asn Ile Tyr
	565	570	575
Phe Ala Thr Asp Ala	Asn Met Leu Leu	Ala Ile Ala Glu	Lys Cys Phe
	580	585	590
Lys Ser Thr Asn Lys	Ile Asn Ala Ile	Phe Ala Gly Lys	Gln Pro Ala
	595	600	605
Ala Thr Trp Ile Thr	Leu Asp Glu Ala	Arg Ala Glu Leu	Glu Ala Gly
	610	615	620
Ala Ala Glu Trp Lys	Trp Ala Ser Asn	Ala Lys Ser Asn	Asp Glu Val
625	630	635	640
Gln Val Val Leu Ala	Ala Ala Gly Asp	Val Pro Thr Gln	Glu Ile Met
	645	650	655
Ala Ala Ser Asp Ala	Leu Asn Lys Met	Gly Ile Lys Phe	Lys Val Val
	660	665	670
Asn Val Val Asp Leu	Ile Lys Leu Gln	Ser Ser Lys Glu	Asn Asp Glu
	675	680	685
Ala Met Ser Asp Glu	Asp Phe Ala Asp	Leu Phe Thr Ala	Asp Lys Pro
	690	695	700
Val Leu Phe Ala Tyr	His Ser Tyr Ala	Gln Asp Val Arg	Gly Leu Ile
705	710	715	720
Tyr Asp Arg Pro Asn	His Asp Asn Phe	Thr Val Val Gly	Tyr Lys Glu
	725	730	735
Gln Gly Ser Thr Thr	Thr Pro Phe Asp	Met Val Arg Val	Asn Asp Met
	740	745	750
Asp Arg Tyr Ala Leu	Gln Ala Lys Ala	Leu Glu Leu Ile	Asp Ala Asp
	755	760	765
Lys Tyr Ala Asp Lys	Ile Asn Glu Leu	Asn Glu Phe Arg	Lys Thr Ala
	770	775	780

195	200	205
Lys Ile Ser Asn Pro Thr Ile Phe Ser Arg Met Ser Asp Glu Glu Ile		
210	215	220
Thr Lys Phe Phe Glu Gly Leu Gly Tyr Ser Pro Arg Phe Ile Glu Asn		
225	230	235
Asp Asp Ile His Asp Tyr Ala Thr Tyr His Gln Leu Ala Ala Asn Ile		
245	250	255
Leu Asp Gln Ala Ile Glu Asp Ile Gln Ala Ile Gln Asn Asp Ala Arg		
260	265	270
Glu Asn Gly Lys Tyr Gln Asp Gly Glu Ile Pro Ala Trp Pro Val Ile		
275	280	285
Ile Ala Arg Leu Pro Lys Gly Trp Gly Gly Pro Thr His Asp Ala Ser		
290	295	300
Asn Asn Pro Ile Glu Asn Ser Phe Arg Ala His Gln Val Pro Leu Pro		
305	310	315
Leu Glu Gln His Asp Leu Ala Thr Leu Pro Glu Phe Glu Asp Trp Met		
325	330	335
Asn Ser Tyr Lys Pro Glu Glu Leu Phe Asn Ala Asp Gly Ser Leu Lys		
340	345	350
Asp Glu Leu Lys Ala Ile Ala Pro Lys Gly Asp Lys Arg Met Ser Ala		
355	360	365
Asn Pro Ile Thr Asn Gly Gly Ala Asp Arg Ser Asp Leu Lys Leu Pro		
370	375	380
Asn Trp Arg Glu Phe Ala Asn Asp Ile Asn Asp Asn Thr Arg Gly Lys		
385	390	395
Glu Phe Ala Asp Ser Lys Arg Asn Met Asp Met Ala Thr Leu Ser Asn		
405	410	415
Tyr Leu Gly Ala Val Ser Gln Leu Asn Pro Thr Arg Phe Arg Phe Phe		
420	425	430
Gly Pro Asp Glu Thr Met Ser Asn Arg Leu Trp Gly Leu Phe Asn Val		
435	440	445
Thr Pro Arg Gln Trp Met Glu Glu Ile Lys Glu Pro Gln Asp Gln Leu		
450	455	460
Leu Ser Pro Thr Gly Arg Ile Ile Asp Ser Gln Leu Ser Glu His Gln		
465	470	475
Ala Glu Gly Trp Leu Glu Gly Tyr Thr Leu Thr Gly Arg Val Gly Ile		
485	490	495
Phe Ala Ser Tyr Glu Ser Phe Leu Arg Val Val Asp Thr Met Val Thr		
500	505	510

Gln His Phe Lys Trp Leu Arg His Ala Ser Glu Gln Ala Trp Arg Asn		
515	520	525
Asp Tyr Pro Ser Leu Asn Leu Ile Ala Thr Ser Thr Ala Phe Gln Gln		
530	535	540
Asp His Asn Gly Tyr Thr His Gln Asp Pro Gly Met Leu Thr His Leu		
545	550	555
Ala Glu Lys Lys Ser Asn Phe Ile Arg Glu Tyr Leu Pro Ala Asp Gly		
	565	570
Asn Ser Leu Leu Ala Val Gln Glu Arg Ala Phe Ser Glu Arg His Lys		
	580	585
Val Asn Leu Leu Ile Ala Ser Lys Gln Pro Arg Gln Gln Trp Phe Thr		
	595	600
Val Glu Glu Ala Glu Val Leu Ala Asn Glu Gly Leu Lys Ile Ile Asp		
610	615	620
Trp Ala Ser Thr Ala Pro Ser Gly Asp Val Asp Ile Thr Phe Ala Ser		
625	630	635
Ala Gly Thr Glu Pro Thr Ile Glu Thr Leu Ala Ala Leu Trp Leu Ile		
	645	650
Asn Gln Ala Phe Pro Asp Val Lys Phe Arg Tyr Val Asn Val Val Glu		
	660	665
Leu Leu Arg Leu Gln Lys Lys Ser Glu Pro Asn Met Asn Asp Glu Arg		
	675	680
Glu Leu Ser Ala Glu Glu Phe Asn Lys Tyr Phe Gln Ala Asp Thr Pro		
690	695	700
Val Ile Phe Gly Phe His Ala Tyr Glu Asn Leu Ile Glu Ser Phe Phe		
705	710	715
Phe Glu Arg Lys Phe Thr Gly Asp Val Tyr Val His Gly Tyr Arg Glu		
	725	730
Asp Gly Asp Ile Thr Thr Thr Tyr Asp Met Arg Val Tyr Ser His Leu		
	740	745
Asp Arg Phe His Gln Ala Lys Glu Ala Ala Glu Ile Leu Ser Ala Asn		
	755	760
Gly Lys Ile Asp Gln Ala Ala Ala Asp Thr Phe Ile Ala Lys Met Asp		
770	775	780
Asp Thr Leu Ala Lys His Phe Gln Val Thr Arg Asn Glu Gly Arg Asp		
785	790	795
Ile Glu Glu Phe Thr Asp Trp Thr Trp Ser Pro Leu Lys		
	805	810

<210> 20

<211> 323

<212> PRT

<213> 枯草芽孢杆菌(Bacillus subtilis)

<220>

<223> 枯草芽孢杆菌磷酸转乙酰酶(Bs_PTA.orf)氨基酸
序列

<400> 20

Met	Ala	Asp	Leu	Phe	Ser	Thr	Val	Gln	Glu	Lys	Val	Ala	Gly	Lys	Asp
1				5					10					15	
Val	Lys	Ile	Val	Phe	Pro	Glu	Gly	Leu	Asp	Glu	Arg	Ile	Leu	Glu	Ala
			20					25					30		
Val	Ser	Lys	Leu	Ala	Gly	Asn	Lys	Val	Leu	Asn	Pro	Ile	Val	Ile	Gly
		35				40					45				
Asn	Glu	Asn	Glu	Ile	Gln	Ala	Lys	Ala	Lys	Glu	Leu	Asn	Leu	Thr	Leu
	50					55				60					
Gly	Gly	Val	Lys	Ile	Tyr	Asp	Pro	His	Thr	Tyr	Glu	Gly	Met	Glu	Asp
65				70						75				80	
Leu	Val	Gln	Ala	Phe	Val	Glu	Arg	Arg	Lys	Gly	Lys	Ala	Thr	Glu	Glu
			85					90					95		
Gln	Ala	Arg	Lys	Ala	Leu	Leu	Asp	Glu	Asn	Tyr	Phe	Gly	Thr	Met	Leu
		100						105				110			
Val	Tyr	Lys	Gly	Leu	Ala	Asp	Gly	Leu	Val	Ser	Gly	Ala	Ala	His	Ser
	115						120				125				
Thr	Ala	Asp	Thr	Val	Arg	Pro	Ala	Leu	Gln	Ile	Ile	Lys	Thr	Lys	Glu
	130					135					140				
Gly	Val	Lys	Lys	Thr	Ser	Gly	Val	Phe	Ile	Met	Ala	Arg	Gly	Glu	Glu
145				150						155				160	
Gln	Tyr	Val	Phe	Ala	Asp	Cys	Ala	Ile	Asn	Ile	Ala	Pro	Asp	Ser	Gln
			165					170				175			
Asp	Leu	Ala	Glu	Ile	Ala	Ile	Glu	Ser	Ala	Asn	Thr	Ala	Lys	Met	Phe
		180					185					190			
Asp	Ile	Glu	Pro	Arg	Val	Ala	Met	Leu	Ser	Phe	Ser	Thr	Lys	Gly	Ser
	195					200					205				
Ala	Lys	Ser	Asp	Glu	Thr	Glu	Lys	Val	Ala	Asp	Ala	Val	Lys	Ile	Ala
	210					215				220					
Lys	Glu	Lys	Ala	Pro	Glu	Leu	Thr	Leu	Asp	Gly	Glu	Phe	Gln	Phe	Asp
225				230						235				240	
Ala	Ala	Phe	Val	Pro	Ser	Val	Ala	Glu	Lys	Lys	Ala	Pro	Asp	Ser	Glu
			245					250					255		

Ile Lys Gly Asp Ala Asn Val Phe Val Phe Pro Ser Leu Glu Ala Gly
 260 265 270
 Asn Ile Gly Tyr Lys Ile Ala Gln Arg Leu Gly Asn Phe Glu Ala Val
 275 280 285
 Gly Pro Ile Leu Gln Gly Leu Asn Met Pro Val Asn Asp Leu Ser Arg
 290 295 300
 Gly Cys Asn Ala Glu Asp Val Tyr Asn Leu Ala Leu Ile Thr Ala Ala
 305 310 315 320
 Gln Ala Leu
 <210> 21
 <211> 325
 <212> PRT
 <213> 植物乳杆菌(Lactobacillus plantarum)
 <220>
 <223> 植物乳杆菌磷酸转乙酰酶(Lpla_PTA.orf)氨基酸
 序列
 <400> 21
 Met Asp Leu Phe Glu Ser Leu Ser Gln Lys Ile Thr Gly Gln Asp Gln
 1 5 10 15
 Thr Ile Val Phe Pro Glu Gly Thr Glu Pro Arg Ile Val Gly Ala Ala
 20 25 30
 Ala Arg Leu Ala Ala Asp Gly Leu Val Lys Pro Ile Val Leu Gly Ala
 35 40 45
 Thr Asp Lys Val Gln Ala Val Ala Lys Asp Leu Asn Ala Asp Leu Thr
 50 55 60
 Gly Val Gln Val Leu Asp Pro Ala Thr Tyr Pro Ala Glu Asp Lys Gln
 65 70 75 80
 Ala Met Leu Asp Ser Leu Val Glu Arg Arg Lys Gly Lys Asn Thr Pro
 85 90 95
 Glu Gln Ala Ala Lys Met Leu Glu Asp Glu Asn Tyr Phe Gly Thr Met
 100 105 110
 Leu Val Tyr Met Gly Lys Ala Asp Gly Met Val Ser Gly Ala Val His
 115 120 125
 Pro Thr Gly Asp Thr Val Arg Pro Ala Leu Gln Ile Ile Lys Thr Lys
 130 135 140
 Pro Gly Ser His Arg Ile Ser Gly Ala Phe Ile Met Gln Lys Gly Glu
 145 150 155 160
 Glu Arg Tyr Val Phe Ala Asp Cys Ala Ile Asn Ile Asp Pro Asp Ala
 165 170 175

Asp Thr Leu Ala Glu Ile Ala Thr Gln Ser Ala Ala Thr Ala Lys Val
 180 185 190
 Phe Asp Ile Asp Pro Lys Val Ala Met Leu Ser Phe Ser Thr Lys Gly
 195 200 205
 Ser Ala Lys Gly Asp Met Val Thr Lys Val Gln Glu Ala Thr Ala Lys
 210 215 220
 Ala Gln Ala Ala Ala Pro Glu Leu Ala Ile Asp Gly Glu Met Gln Phe
 225 230 235 240
 Asp Ala Ala Phe Val Glu Lys Val Gly Leu Gln Lys Ala Pro Gly Ser
 245 250 255
 Lys Val Ala Gly His Ala Asn Val Phe Val Phe Pro Glu Leu Gln Ser
 260 265 270
 Gly Asn Ile Gly Tyr Lys Ile Ala Gln Arg Phe Gly His Phe Glu Ala
 275 280 285
 Val Gly Pro Val Leu Gln Gly Leu Asn Lys Pro Val Ser Asp Leu Ser
 290 295 300
 Arg Gly Cys Ser Glu Glu Asp Val Tyr Lys Val Ala Ile Ile Thr Ala
 305 310 315 320
 Ala Gln Gly Leu Ala
 325

<210> 22

<211> 556

<212> PRT

<213> 青春双歧杆菌(Bifidobacterium adoloscentis)

<220>

<223> 青春双歧杆菌磷酸转乙酰酶(Bado_PTA.orf)氨基酸
序列

<400> 22

Met Ser Phe Thr Ser Val Thr Ile Ile Ser Pro Glu Ala Ala Asn Gly
 1 5 10 15
 Arg Asn Val Val Ala Leu Gly Val Thr Lys Thr Leu Ala Ala Ala Gly
 20 25 30
 Lys Thr Gly Val Phe Arg Pro Ala Val Cys Arg Lys Asp Thr Phe Thr
 35 40 45
 Asp Val Leu Ile Glu Ala Ser Asn Ala Gly Leu Ser Arg Glu Gln Ser
 50 55 60
 Val Gly Val Cys Pro Lys Arg Ala Arg Asn Asp Lys Glu Gly Ser Arg
 65 70 75 80
 Ala Asp Ile Val Ala Ala Tyr Thr Gln Ala Val Glu Thr Ala Arg Pro

85	90	95
Asp Ala Met Val Ile Val Gly Thr	Asp Arg Ser Ala Val Asn Asp Pro	
100	105	110
Ala Met Phe Ser Phe Asn Ala Asp Val Ala Ala Asp Leu Gln Ser Pro		
115	120	125
Val Leu Leu Ala Val Cys Thr Ile Glu Arg Thr Pro Glu Gln Val Lys		
130	135	140
Ser Thr Val Glu Ala Ser Thr Lys Val Ile Glu Asp Ala Gly Ser Lys		
145	150	155
Val Val Gly Val Phe Ile Thr Gly Cys Asp Asp Thr Gln Pro Asn Pro		
165	170	175
Leu Lys Ala Cys Phe Val Asp Tyr Pro Val Pro Val Trp Thr Leu Pro		
180	185	190
Ala Val Asp Phe Asn Asp Asp Asp Ala Ile Ser Lys Ala Asp Glu Ala		
195	200	205
Phe Ala Thr Asn Val Asp Ala Val Glu Leu Thr Val Ala Leu Glu Ser		
210	215	220
Pro Phe Asp Ala Pro Thr Thr Pro Tyr Ala Phe Gln Tyr Gly Leu Leu		
225	230	235
Gly Lys Ala Lys Ala Asp Lys Lys Thr Ile Val Leu Pro Glu Gly Asn		
245	250	255
Glu Asp Arg Ile Ile Lys Ala Ala Asp Tyr Leu Leu Glu Arg Asp Ile		
260	265	270
Val Asp Leu Ile Ile Val Gly Asp Glu Asn Ala Ile Leu Ala Arg Gly		
275	280	285
Gln Glu Leu Gly Leu Lys Ser Leu Gly Lys Ala Lys Phe Gln Ala Lys		
290	295	300
Asp Asp Glu Thr Val Leu Glu Pro Met Val Ala Lys Leu Cys Glu Leu		
305	310	315
Arg Ala Lys Lys Gly Met Thr Glu Glu Gln Ala Arg Lys Gln Leu Ala		
325	330	335
Asp Asp Ser Tyr Phe Gly Thr Met Leu Val Val Met Gly Met Ala Asp		
340	345	350
Gly Leu Val Ser Gly Ser Val Asn Ser Thr Ala Asn Thr Val Arg Pro		
355	360	365
Ala Leu Gln Val Ile Lys Thr Lys Pro Gly Thr Ser Leu Val Ser Gly		
370	375	380
Ala Phe Leu Met Cys Phe Lys Asp His Ala Ala Val Phe Ala Asp Cys		
385	390	395
		400

Ala	Ile	Asn	Leu	Asn	Pro	Asn	Ala	Glu	Gln	Leu	Ala	Glu	Ile	Ala	Ile	
				405					410					415		
Gln	Ser	Ala	Glu	Thr	Ala	Lys	Ala	Phe	Gly	Leu	Glu	Pro	Lys	Val	Gly	
				420					425					430		
Met	Leu	Ser	Tyr	Ser	Thr	Leu	Gly	Ser	Gly	Lys	Gly	Pro	Asp	Val	Asp	
				435					440					445		
Leu	Val	Glu	Glu	Ala	Thr	Thr	Ile	Val	Lys	Asp	Lys	Ala	Pro	Asp	Leu	
				450					455					460		
Ala	Val	Val	Gly	Ser	Ile	Gln	Phe	Asp	Ala	Ala	Trp	Ser	Pro	Thr	Val	
				465					470					475		
Ala	Ala	Thr	Lys	Ala	Lys	Gly	Asp	Pro	Val	Ala	Gly	His	Val	Asn	Val	
				485					490					495		
Phe	Val	Phe	Pro	Asp	Leu	Cys	Ala	Gly	Asn	Ile	Ala	Tyr	Lys	Ala	Val	
				500					505					510		
Gln	Arg	Ser	Ser	Gly	Ala	Ala	Ala	Val	Gly	Pro	Val	Leu	Gln	Gly	Leu	
				515					520					525		
Asn	Arg	Pro	Val	Asn	Asp	Leu	Ser	Arg	Gly	Ala	Thr	Val	Gln	Asp	Ile	
				530					535					540		
Ile	Asn	Thr	Ile	Ala	Leu	Thr	Ala	Ile	Glu	Ala	Gln					
				545					550					555		

<210> 23

<211> 333

<212> PRT

<213> 嗜热毁丝霉 (*Methosarcina thermophila*)

<220>

<223> 嗜热毁丝霉磷酸转乙酰酶 (Mthe_PTA.orf) 氨基酸
序列

<400> 23

Met	Val	Thr	Phe	Leu	Glu	Lys	Ile	Ser	Glu	Arg	Ala	Lys	Lys	Leu	Asn	
1				5				10				15				
Lys	Thr	Ile	Ala	Leu	Pro	Glu	Thr	Glu	Asp	Ile	Arg	Thr	Leu	Gln	Ala	
				20					25					30		
Ala	Ala	Lys	Ile	Leu	Glu	Arg	Gly	Ile	Ala	Asp	Ile	Val	Leu	Val	Gly	
				35					40					45		
Asn	Glu	Ala	Asp	Ile	Lys	Ala	Leu	Ala	Gly	Asp	Leu	Asp	Leu	Ser	Lys	
				50					55					60		
Ala	Lys	Ile	Val	Asp	Pro	Lys	Thr	Tyr	Glu	Lys	Lys	Asp	Glu	Tyr	Ile	
				65					70					75		
Asn	Ala	Phe	Tyr	Glu	Leu	Arg	Lys	His	Lys	Gly	Ile	Thr	Leu	Glu	Asn	

				85				90				95			
Ala	Ala	Glu	Ile	Met	Ser	Asp	Tyr	Val	Tyr	Phe	Ala	Val	Met	Met	Ala
				100				105				110			
Lys	Leu	Gly	Glu	Val	Asp	Gly	Val	Val	Ser	Gly	Ala	Ala	His	Ser	Ser
				115				120				125			
Ser	Asp	Thr	Leu	Arg	Pro	Ala	Val	Gln	Ile	Val	Lys	Thr	Ala	Lys	Gly
				130				135				140			
Ala	Ala	Leu	Ala	Ser	Ala	Phe	Phe	Ile	Ile	Ser	Val	Pro	Asp	Cys	Glu
145				150				155				160			
Tyr	Gly	Ser	Asp	Gly	Thr	Phe	Leu	Phe	Ala	Asp	Ser	Gly	Met	Val	Glu
				165				170				175			
Met	Pro	Ser	Val	Glu	Asp	Val	Ala	Asn	Ile	Ala	Val	Ile	Ser	Ala	Lys
				180				185				190			
Thr	Phe	Glu	Leu	Leu	Val	Gln	Asp	Val	Pro	Lys	Val	Ala	Met	Leu	Ser
				195				200				205			
Tyr	Ser	Thr	Lys	Gly	Ser	Ala	Lys	Ser	Lys	Leu	Thr	Glu	Ala	Thr	Ile
				210				215				220			
Ala	Ser	Thr	Lys	Leu	Ala	Gln	Glu	Leu	Ala	Pro	Asp	Ile	Ala	Ile	Asp
225				230				235				240			
Gly	Glu	Leu	Gln	Val	Asp	Ala	Ala	Ile	Val	Pro	Lys	Val	Ala	Ala	Ser
				245				250				255			
Lys	Ala	Pro	Gly	Ser	Pro	Val	Ala	Gly	Lys	Ala	Asn	Val	Phe	Ile	Phe
				260				265				270			
Pro	Asp	Leu	Asn	Cys	Gly	Asn	Ile	Ala	Tyr	Lys	Ile	Ala	Gln	Arg	Leu
				275				280				285			
Ala	Lys	Ala	Glu	Ala	Tyr	Gly	Pro	Ile	Thr	Gln	Gly	Leu	Ala	Lys	Pro
				290				295				300			
Ile	Asn	Asp	Leu	Ser	Arg	Gly	Cys	Ser	Asp	Glu	Asp	Ile	Val	Gly	Ala
305				310				315				320			
Val	Ala	Ile	Thr	Cys	Val	Gln	Ala	Ala	Ala	Gln	Asp	Lys			
				325				330							

<210> 24

<211> 409

<212> PRT

<213> 青春双歧杆菌(Bifidobacterium adolescentis)

<220>

<223> 青春双歧杆菌乙酸激酶(Bado_ACK)氨基酸序列

<400> 24

Met Ala Lys Thr Val Leu Val Ile Asn Ser Gly Ser Ser Ser Ile Lys

1					5					10					15				
Tyr	Gln	Leu	Val	Asp	Leu	Glu	Thr	Gly	Glu	Gly	Ile	Ala	Ser	Gly	Leu				
				20				25				30							
Val	Glu	Lys	Ile	Gly	Glu	Pro	Val	Asp	Gly	His	Tyr	Lys	His	Glu	Tyr				
				35				40				45							
Asn	Gly	Glu	Lys	His	Glu	Leu	Glu	Glu	Pro	Ile	His	Asp	His	Glu	Gln				
				50				55				60							
Gly	Leu	Lys	Arg	Val	Leu	Gly	Phe	Phe	Asp	Glu	Phe	Gly	Pro	Lys	Leu				
65					70				75				80						
Ala	Asp	Ala	Gly	Ile	Val	Ala	Val	Gly	His	Arg	Val	Val	Gln	Gly	Gly				
				85				90				95							
Ser	Ile	Phe	Pro	Lys	Pro	Ala	Leu	Val	Asn	Asp	Lys	Thr	Ile	Gly	Gln				
				100				105				110							
Val	Lys	Asp	Leu	Ala	Val	Leu	Ala	Pro	Leu	His	Asn	Gly	Pro	Glu	Ala				
				115				120				125							
Lys	Gly	Ala	Glu	Val	Met	Arg	Ser	Leu	Leu	Pro	Asp	Val	Pro	Gln	Ile				
				130				135				140							
Phe	Val	Phe	Asp	Ser	Ser	Phe	Phe	Phe	Gln	Leu	Pro	Lys	Ala	Ser	Ser				
145					150				155				160						
Thr	Tyr	Ala	Leu	Asn	Lys	Glu	Val	Ala	Gln	Gln	Tyr	His	Ile	Arg	Arg				
				165				170				175							
Tyr	Gly	Ala	His	Gly	Thr	Ser	His	Glu	Phe	Ile	Ser	Ser	Val	Val	Pro				
				180				185				190							
Ser	Val	Ile	Gly	Lys	Pro	Ala	Glu	Gly	Leu	Lys	Gln	Ile	Val	Leu	His				
				195				200				205							
Ile	Gly	Asn	Gly	Ala	Ser	Ala	Ser	Ala	Glu	Ile	Ser	Gly	Lys	Pro	Val				
				210				215				220							
Glu	Thr	Ser	Met	Gly	Leu	Thr	Pro	Leu	Glu	Gly	Leu	Val	Met	Gly	Gly				
225					230				235				240						
Arg	Thr	Gly	Asp	Ile	Asp	Pro	Ala	Val	Val	Phe	His	Leu	Ile	Arg	Asn				
				245				250				255							
Ala	His	Met	Ser	Val	Asp	Glu	Leu	Asp	Thr	Leu	Phe	Asn	Lys	Arg	Ser				
				260				265				270							
Gly	Met	Met	Gly	Leu	Thr	Gly	Phe	Gly	Asp	Leu	Arg	Glu	Val	His	Arg				
				275				280				285							
Leu	Val	Glu	Glu	Gly	Asn	Glu	Asp	Ala	Lys	Leu	Ala	Leu	Asp	Ile	Tyr				
				290				295				300							
Val	His	Arg	Ile	Val	Gly	Tyr	Ile	Gly	Asn	Tyr	Thr	Ala	Gln	Met	Gly				
305					310				315				320						

Gly Val Asp Val Ile Thr Phe Thr Ala Gly Val Gly Glu Asn Asp Asp
 325 330 335
 Val Val Arg Lys Met Val Cys Asp Lys Leu Ala Pro Phe Gly Val Lys
 340 345 350
 Leu Asp Glu Glu Lys Asn Ala Thr Arg Ser Lys Glu Pro Arg Ile Ile
 355 360 365
 Ser Thr Pro Asp Ser Ala Val Thr Ile Cys Val Ile Pro Thr Asn Glu
 370 375 380
 Glu Leu Ala Ile Ala Arg Lys Ser Ala Ala Ile Ala Glu Glu Gly Lys
 385 390 395 400
 Asp Ser Tyr Gly Asn Val Phe Ser Lys
 405

<210> 25

<211> 420

<212> PRT

<213> 构巢曲霉(Aspergillus nidulans)

<220>

<223> 构巢曲霉ACK(Ani_ACK)氨基酸序列

<400> 25

Met Pro Arg Lys Ser Ile Leu Ser Val Asn Ala Gly Ser Ser Ser Val
 1 5 10 15
 Lys Ile Thr Phe Tyr Ser Tyr Thr Lys Thr Pro Ser Val Ile Ala Thr
 20 25 30
 Ala Gln Val Ser Gly Ile Thr Ala Pro Pro Ala Thr Phe Lys Tyr Ser
 35 40 45
 Val Gly Ser Lys Gln Lys Lys Glu Glu Leu Lys Glu Lys Ile Ser Ser
 50 55 60
 Gly Pro Asp Ala Phe Lys Leu Leu Leu His Arg Cys Phe Thr Asp Ser
 65 70 75 80
 Asp Leu Lys Asp Val Ala Ser Ala Asp Asp Leu Ala Tyr Ile Cys His
 85 90 95
 Arg Val Val His Gly Gly Asp Phe Glu Ser Pro Val Val Ile Asn Glu
 100 105 110
 Glu Thr Tyr His Gln Leu Glu Asp Leu Glu Asp Leu Ala Pro Leu His
 115 120 125
 Asn Phe Ala Ala Leu Glu Ile Val Arg Leu Cys Lys Lys Glu Leu Pro
 130 135 140
 Asn Val Gln Ser Ile Thr Phe Phe Asp Ser Ser Phe His Lys Ser Leu
 145 150 155 160

Pro	Pro	Tyr	Val	Lys	Thr	Tyr	Pro	Ile	Asp	Gln	Glu	Thr	Ala	Arg	Arg			
																165	170	175
Asn	Lys	Leu	Arg	Lys	Tyr	Gly	Phe	His	Gly	Ile	Ser	Tyr	Ser	Phe	Ile			
																180	185	190
Leu	Arg	Ser	Val	Ala	Glu	Tyr	Leu	Asn	Lys	Pro	Val	Glu	Lys	Thr	Ser			
																195	200	205
Leu	Ile	Ala	Leu	His	Ile	Gly	Ser	Gly	Ala	Ser	Val	Cys	Ala	Ile	Lys			
																210	215	220
Asp	Gly	Lys	Ser	Ile	Asp	Thr	Ser	Met	Gly	Leu	Thr	Pro	Leu	Ala	Gly			
																225	230	235
Leu	Pro	Gly	Ala	Thr	Arg	Ser	Gly	Asp	Ile	Asp	Pro	Ser	Leu	Val	Phe			
																245	250	255
His	Tyr	Thr	Asn	Glu	Ala	Gly	Lys	Leu	Ser	Pro	Ala	Ser	Thr	Lys	Glu			
																260	265	270
Met	His	Ile	Ser	Thr	Ala	Glu	Glu	Ile	Leu	Asn	Lys	Lys	Ser	Gly	Trp			
																275	280	285
Lys	Val	Leu	Thr	Gly	Thr	Thr	Asp	Phe	Ser	Gln	Ile	Ala	Val	Glu	Asp			
																290	295	300
Pro	Pro	Ser	Glu	Gln	His	Lys	Leu	Ala	Phe	Asp	Ile	Leu	Val	Asp	Arg			
																305	310	315
Ile	Val	Gly	Tyr	Ile	Gly	Asn	Tyr	Tyr	Val	Lys	Leu	Asp	Gly	Gln	Val			
																325	330	335
Glu	Gly	Ile	Val	Phe	Ala	Gly	Gly	Ile	Gly	Glu	Lys	Ser	Ala	Leu	Leu			
																340	345	350
Arg	Lys	Ala	Val	Ile	Glu	Gln	Thr	Arg	Cys	Leu	Gly	Phe	Ala	Val	Asp			
																355	360	365
Pro	Glu	Lys	Asn	Gln	His	Gly	Pro	Gly	Asp	Asp	Glu	Thr	Val	Val	Asp			
																370	375	380
Ile	Thr	Ala	Ser	Gly	Arg	Ser	Asp	Val	Lys	Arg	Val	Phe	Ile	Cys	Gln			
																385	390	395
Thr	Asp	Glu	Gln	Phe	Glu	Met	Ala	Tyr	Asn	Cys	Thr	Lys	Thr	Gln	Gly			
																405	410	415
Leu	Asp	Lys	Gln															
																420		

<210> 26
 <211> 506
 <212> PRT
 <213> 酿酒酵母(Saccharomyces cerevisiae)
 <220>

<223> 酿酒酵母 (ALDH2) 醛脱氢酶氨基酸

序列

<400> 26

Met	Pro	Thr	Leu	Tyr	Thr	Asp	Ile	Glu	Ile	Pro	Gln	Leu	Lys	Ile	Ser	1	5	10	15
Leu	Lys	Gln	Pro	Leu	Gly	Leu	Phe	Ile	Asn	Asn	Glu	Phe	Cys	Pro	Ser	20	25	30	
Ser	Asp	Gly	Lys	Thr	Ile	Glu	Thr	Val	Asn	Pro	Ala	Thr	Gly	Glu	Pro	35	40	45	
Ile	Thr	Ser	Phe	Gln	Ala	Ala	Asn	Glu	Lys	Asp	Val	Asp	Lys	Ala	Val	50	55	60	
Lys	Ala	Ala	Arg	Ala	Ala	Phe	Asp	Asn	Val	Trp	Ser	Lys	Thr	Ser	Ser	65	70	75	80
Glu	Gln	Arg	Gly	Ile	Tyr	Leu	Ser	Asn	Leu	Leu	Lys	Leu	Ile	Glu	Glu	85	90	95	
Glu	Gln	Asp	Thr	Leu	Ala	Ala	Leu	Glu	Thr	Leu	Asp	Ala	Gly	Lys	Pro	100	105	110	
Tyr	His	Ser	Asn	Ala	Lys	Gly	Asp	Leu	Ala	Gln	Ile	Leu	Gln	Leu	Thr	115	120	125	
Arg	Tyr	Phe	Ala	Gly	Ser	Ala	Asp	Lys	Phe	Asp	Lys	Gly	Ala	Thr	Ile	130	135	140	
Pro	Leu	Thr	Phe	Asn	Lys	Phe	Ala	Tyr	Thr	Leu	Lys	Val	Pro	Phe	Gly	145	150	155	160
Val	Val	Ala	Gln	Ile	Val	Pro	Trp	Asn	Tyr	Pro	Leu	Ala	Met	Ala	Cys	165	170	175	
Trp	Lys	Leu	Gln	Gly	Ala	Leu	Ala	Ala	Gly	Asn	Thr	Val	Ile	Ile	Lys	180	185	190	
Pro	Ala	Glu	Asn	Thr	Ser	Leu	Ser	Leu	Leu	Tyr	Phe	Ala	Thr	Leu	Ile	195	200	205	
Lys	Lys	Ala	Gly	Phe	Pro	Pro	Gly	Val	Val	Asn	Ile	Val	Pro	Gly	Tyr	210	215	220	
Gly	Ser	Leu	Val	Gly	Gln	Ala	Leu	Ala	Ser	His	Met	Asp	Ile	Asp	Lys	225	230	235	240
Ile	Ser	Phe	Thr	Gly	Ser	Thr	Lys	Val	Gly	Gly	Phe	Val	Leu	Glu	Ala	245	250	255	
Ser	Gly	Gln	Ser	Asn	Leu	Lys	Asp	Val	Thr	Leu	Glu	Cys	Gly	Gly	Lys	260	265	270	
Ser	Pro	Ala	Leu	Val	Phe	Glu	Asp	Ala	Asp	Leu	Asp	Lys	Ala	Ile	Asp	275	280	285	

Trp Ile Ala Ala Gly Ile Phe Tyr Asn Ser Gly Gln Asn Cys Thr Ala															
290															
Asn Ser Arg Val Tyr Val Gln Ser Ser Ile Tyr Asp Lys Phe Val Glu															
305															
Lys Phe Lys Glu Thr Ala Lys Lys Glu Trp Asp Val Ala Gly Lys Phe															
Asp Pro Phe Asp Glu Lys Cys Ile Val Gly Pro Val Ile Ser Ser Thr															
Gln Tyr Asp Arg Ile Lys Ser Tyr Ile Glu Arg Gly Lys Arg Glu Glu															
Lys Leu Asp Met Phe Gln Thr Ser Glu Phe Pro Ile Gly Gly Ala Lys															
Gly Tyr Phe Ile Pro Pro Thr Ile Phe Thr Asp Val Pro Gln Thr Ser															
Lys Leu Leu Gln Asp Glu Ile Phe Gly Pro Val Val Val Val Ser Lys															
Phe Thr Asn Tyr Asp Asp Ala Leu Lys Leu Ala Asn Asp Thr Cys Tyr															
Gly Leu Ala Ser Ala Val Phe Thr Lys Asp Val Lys Lys Ala His Met															
Phe Ala Arg Asp Ile Lys Ala Gly Thr Val Trp Ile Asn Ser Ser Asn															
Asp Glu Asp Val Thr Val Pro Phe Gly Gly Phe Lys Met Ser Gly Ile															
Gly Arg Glu Leu Gly Gln Ser Gly Val Asp Thr Tyr Leu Gln Thr Lys															
Ala Val His Ile Asn Leu Ser Leu Asp Asn															
<210> 27															
<211> 506															
<212> PRT															
<213> 酿酒酵母(Saccharomyces cerevisiae)															
<220>															
<223> 酿酒酵母(ALDH3)醛脱氢酶氨基酸															
序列															
<400> 27															
Met Pro Thr Leu Tyr Thr Asp Ile Glu Ile Pro Gln Leu Lys Ile Ser															
1															
Leu Lys Gln Pro Leu Gly Leu Phe Ile Asn Asn Glu Phe Cys Pro Ser															

20	25	30
Ser Asp Gly Lys Thr Ile Glu Thr Val Asn Pro Ala Thr Gly Glu Pro		
35	40	45
Ile Thr Ser Phe Gln Ala Ala Asn Glu Lys Asp Val Asp Lys Ala Val		
50	55	60
Lys Ala Ala Arg Ala Ala Phe Asp Asn Val Trp Ser Lys Thr Ser Ser		
65	70	75
Glu Gln Arg Gly Ile Tyr Leu Ser Asn Leu Leu Lys Leu Ile Glu Glu		
85	90	95
Glu Gln Asp Thr Leu Ala Ala Leu Glu Thr Leu Asp Ala Gly Lys Pro		
100	105	110
Phe His Ser Asn Ala Lys Gln Asp Leu Ala Gln Ile Ile Glu Leu Thr		
115	120	125
Arg Tyr Tyr Ala Gly Ala Val Asp Lys Phe Asn Met Gly Glu Thr Ile		
130	135	140
Pro Leu Thr Phe Asn Lys Phe Ala Tyr Thr Leu Lys Val Pro Phe Gly		
145	150	155
Val Val Ala Gln Ile Val Pro Trp Asn Tyr Pro Leu Ala Met Ala Cys		
165	170	175
Arg Lys Met Gln Gly Ala Leu Ala Ala Gly Asn Thr Val Ile Ile Lys		
180	185	190
Pro Ala Glu Asn Thr Ser Leu Ser Leu Leu Tyr Phe Ala Thr Leu Ile		
195	200	205
Lys Lys Ala Gly Phe Pro Pro Gly Val Val Asn Val Ile Pro Gly Tyr		
210	215	220
Gly Ser Val Val Gly Lys Ala Leu Gly Thr His Met Asp Ile Asp Lys		
225	230	235
Ile Ser Phe Thr Gly Ser Thr Lys Val Gly Gly Ser Val Leu Glu Ala		
245	250	255
Ser Gly Gln Ser Asn Leu Lys Asp Ile Thr Leu Glu Cys Gly Gly Lys		
260	265	270
Ser Pro Ala Leu Val Phe Glu Asp Ala Asp Leu Asp Lys Ala Ile Glu		
275	280	285
Trp Val Ala Asn Gly Ile Phe Phe Asn Ser Gly Gln Ile Cys Thr Ala		
290	295	300
Asn Ser Arg Val Tyr Val Gln Ser Ser Ile Tyr Asp Lys Phe Val Glu		
305	310	315
Lys Phe Lys Glu Thr Ala Lys Lys Glu Trp Asp Val Ala Gly Lys Phe		
325	330	335

Asp Pro Phe Asp Glu Lys Cys Ile Val Gly Pro Val Ile Ser Ser Thr
 340 345 350
 Gln Tyr Asp Arg Ile Lys Ser Tyr Ile Glu Arg Gly Lys Lys Glu Glu
 355 360 365
 Lys Leu Asp Met Phe Gln Thr Ser Glu Phe Pro Ile Gly Gly Ala Lys
 370 375 380
 Gly Tyr Phe Ile Pro Pro Thr Ile Phe Thr Asp Val Pro Glu Thr Ser
 385 390 395 400
 Lys Leu Leu Arg Asp Glu Ile Phe Gly Pro Val Val Val Val Ser Lys
 405 410 415
 Phe Thr Asn Tyr Asp Asp Ala Leu Lys Leu Ala Asn Asp Thr Cys Tyr
 420 425 430
 Gly Leu Ala Ser Ala Val Phe Thr Lys Asp Val Lys Lys Ala His Met
 435 440 445
 Phe Ala Arg Asp Ile Lys Ala Gly Thr Val Trp Ile Asn Gln Thr Asn
 450 455 460
 Gln Glu Glu Ala Lys Val Pro Phe Gly Gly Phe Lys Met Ser Gly Ile
 465 470 475 480
 Gly Arg Glu Ser Gly Asp Thr Gly Val Asp Asn Tyr Leu Gln Ile Lys
 485 490 495
 Ser Val His Val Asp Leu Ser Leu Asp Lys
 500 505

<210> 28

<211> 1038

<212> PRT

<213> 酿酒酵母 (*Saccharomyces cerevisiae*)

<220>

<223> 酿酒酵母 (ALDH4) 醛脱氢酶氨基酸

序列

<400> 28

Met Phe Ser Arg Ser Thr Leu Cys Leu Lys Thr Ser Ala Ser Ser Ile
 1 5 10 15
 Gly Arg Leu Gln Leu Arg Tyr Phe Ser His Leu Pro Met Thr Val Pro
 20 25 30
 Ile Lys Leu Pro Asn Gly Leu Glu Tyr Glu Gln Pro Thr Gly Leu Phe
 35 40 45
 Ile Asn Asn Lys Phe Val Pro Ser Lys Gln Asn Lys Thr Phe Glu Val
 50 55 60
 Ile Asn Pro Ser Thr Glu Glu Glu Ile Cys His Ile Tyr Glu Gly Arg

65	70	75	80
Glu Asp Asp Val	Glu Glu Ala Val	Gln Ala Ala Asp	Arg Ala Phe Ser
85	90	95	
Asn Gly Ser Trp	Asn Gly Ile Asp	Pro Ile Asp Arg	Gly Lys Ala Leu
100	105	110	
Tyr Arg Leu Ala	Glu Leu Ile Glu	Gln Asp Lys Asp	Val Ile Ala Ser
115	120	125	
Ile Glu Thr Leu	Asp Asn Gly Lys	Ala Ile Ser Ser	Ser Arg Gly Asp
130	135	140	
Val Asp Leu Val	Ile Asn Tyr Leu	Lys Ser Ser Ala	Gly Phe Ala Asp
145	150	155	160
Lys Ile Asp Gly	Arg Met Ile Asp	Thr Gly Arg Thr	His Phe Ser Tyr
165	170	175	
Thr Lys Arg Gln	Pro Leu Gly Val	Cys Gly Gln Ile	Ile Pro Trp Asn
180	185	190	
Phe Pro Leu Leu	Met Trp Ala Trp	Lys Ile Ala Pro	Ala Leu Val Thr
195	200	205	
Gly Asn Thr Val	Val Leu Lys Thr	Ala Glu Ser Thr	Pro Leu Ser Ala
210	215	220	
Leu Tyr Val Ser	Lys Tyr Ile Pro	Gln Ala Gly Ile	Pro Pro Gly Val
225	230	235	240
Ile Asn Ile Val	Ser Gly Phe Gly	Lys Ile Val Gly	Glu Ala Ile Thr
245	250	255	
Asn His Pro Lys	Ile Lys Lys Val	Ala Phe Thr Gly	Ser Thr Ala Thr
260	265	270	
Gly Arg His Ile	Tyr Gln Ser Ala	Ala Ala Gly Leu	Lys Lys Val Thr
275	280	285	
Leu Glu Leu Gly	Gly Lys Ser Pro	Asn Ile Val Phe	Ala Asp Ala Glu
290	295	300	
Leu Lys Lys Ala	Val Gln Asn Ile	Ile Leu Gly Ile	Tyr Tyr Asn Ser
305	310	315	320
Gly Glu Val Cys	Cys Ala Gly Ser	Arg Val Tyr Val	Glu Glu Ser Ile
325	330	335	
Tyr Asp Lys Phe	Ile Glu Glu Phe	Lys Ala Ala Ser	Glu Ser Ile Lys
340	345	350	
Val Gly Asp Pro	Phe Asp Glu Ser	Thr Phe Gln Gly	Ala Gln Thr Ser
355	360	365	
Gln Met Gln Leu	Asn Lys Ile Leu	Lys Tyr Val Asp	Ile Gly Lys Asn
370	375	380	

Glu Gly Ala Thr Leu Ile Thr Gly Gly Glu Arg Leu Gly Ser Lys Gly			
385	390	395	400
Tyr Phe Ile Lys Pro Thr Val Phe Gly Asp Val Lys Glu Asp Met Arg			
	405	410	415
Ile Val Lys Glu Glu Ile Phe Gly Pro Val Val Thr Val Thr Lys Phe			
	420	425	430
Lys Ser Ala Asp Glu Val Ile Asn Met Ala Asn Asp Ser Glu Tyr Gly			
	435	440	445
Leu Ala Ala Gly Ile His Thr Ser Asn Ile Asn Thr Ala Leu Lys Val			
	450	455	460
Ala Asp Arg Val Asn Ala Gly Thr Val Trp Ile Asn Thr Tyr Asn Asp			
465	470	475	480
Phe His His Ala Val Pro Phe Gly Gly Phe Asn Ala Ser Gly Leu Gly			
	485	490	495
Arg Glu Met Ser Val Asp Ala Leu Gln Asn Tyr Leu Gln Val Lys Ala			
	500	505	510
Val Arg Ala Lys Leu Asp Glu Met Phe Ser Arg Ser Thr Leu Cys Leu			
	515	520	525
Lys Thr Ser Ala Ser Ser Ile Gly Arg Leu Gln Leu Arg Tyr Phe Ser			
	530	535	540
His Leu Pro Met Thr Val Pro Ile Lys Leu Pro Asn Gly Leu Glu Tyr			
545	550	555	560
Glu Gln Pro Thr Gly Leu Phe Ile Asn Asn Lys Phe Val Pro Ser Lys			
	565	570	575
Gln Asn Lys Thr Phe Glu Val Ile Asn Pro Ser Thr Glu Glu Glu Ile			
	580	585	590
Cys His Ile Tyr Glu Gly Arg Glu Asp Asp Val Glu Glu Ala Val Gln			
	595	600	605
Ala Ala Asp Arg Ala Phe Ser Asn Gly Ser Trp Asn Gly Ile Asp Pro			
	610	615	620
Ile Asp Arg Gly Lys Ala Leu Tyr Arg Leu Ala Glu Leu Ile Glu Gln			
625	630	635	640
Asp Lys Asp Val Ile Ala Ser Ile Glu Thr Leu Asp Asn Gly Lys Ala			
	645	650	655
Ile Ser Ser Ser Arg Gly Asp Val Asp Leu Val Ile Asn Tyr Leu Lys			
	660	665	670
Ser Ser Ala Gly Phe Ala Asp Lys Ile Asp Gly Arg Met Ile Asp Thr			
	675	680	685
Gly Arg Thr His Phe Ser Tyr Thr Lys Arg Gln Pro Leu Gly Val Cys			

690	695	700
Gly Gln Ile Ile Pro Trp Asn Phe Pro Leu Leu Met Trp Ala Trp Lys		
705	710	715
Ile Ala Pro Ala Leu Val Thr Gly Asn Thr Val Val Leu Lys Thr Ala		
725	730	735
Glu Ser Thr Pro Leu Ser Ala Leu Tyr Val Ser Lys Tyr Ile Pro Gln		
740	745	750
Ala Gly Ile Pro Pro Gly Val Ile Asn Ile Val Ser Gly Phe Gly Lys		
755	760	765
Ile Val Gly Glu Ala Ile Thr Asn His Pro Lys Ile Lys Lys Val Ala		
770	775	780
Phe Thr Gly Ser Thr Ala Thr Gly Arg His Ile Tyr Gln Ser Ala Ala		
785	790	795
Ala Gly Leu Lys Lys Val Thr Leu Glu Leu Gly Gly Lys Ser Pro Asn		
805	810	815
Ile Val Phe Ala Asp Ala Glu Leu Lys Lys Ala Val Gln Asn Ile Ile		
820	825	830
Leu Gly Ile Tyr Tyr Asn Ser Gly Glu Val Cys Cys Ala Gly Ser Arg		
835	840	845
Val Tyr Val Glu Glu Ser Ile Tyr Asp Lys Phe Ile Glu Glu Phe Lys		
850	855	860
Ala Ala Ser Glu Ser Ile Lys Val Gly Asp Pro Phe Asp Glu Ser Thr		
865	870	875
Phe Gln Gly Ala Gln Thr Ser Gln Met Gln Leu Asn Lys Ile Leu Lys		
885	890	895
Tyr Val Asp Ile Gly Lys Asn Glu Gly Ala Thr Leu Ile Thr Gly Gly		
900	905	910
Glu Arg Leu Gly Ser Lys Gly Tyr Phe Ile Lys Pro Thr Val Phe Gly		
915	920	925
Asp Val Lys Glu Asp Met Arg Ile Val Lys Glu Glu Ile Phe Gly Pro		
930	935	940
Val Val Thr Val Thr Lys Phe Lys Ser Ala Asp Glu Val Ile Asn Met		
945	950	955
Ala Asn Asp Ser Glu Tyr Gly Leu Ala Ala Gly Ile His Thr Ser Asn		
965	970	975
Ile Asn Thr Ala Leu Lys Val Ala Asp Arg Val Asn Ala Gly Thr Val		
980	985	990
Trp Ile Asn Thr Tyr Asn Asp Phe His His Ala Val Pro Phe Gly Gly		
995	1000	1005

Phe Asn Ala Ser Gly Leu Gly Arg Glu Met Ser Val Asp Ala Leu Gln
 1010 1015 1020
 Asn Tyr Leu Gln Val Lys Ala Val Arg Ala Lys Leu Asp Glu
 1025 1030 1035
 <210> 29
 <211> 520
 <212> PRT
 <213> 酿酒酵母 (*Saccharomyces cerevisiae*)
 <220>
 <223> 酿酒酵母 (ALDH5) 醛脱氢酶氨基酸
 序列
 <400> 29
 Met Leu Ser Arg Thr Arg Ala Ala Ala Pro Asn Ser Arg Ile Phe Thr
 1 5 10 15
 Arg Ser Leu Leu Arg Leu Tyr Ser Gln Ala Pro Leu Arg Val Pro Ile
 20 25 30
 Thr Leu Pro Asn Gly Phe Thr Tyr Glu Gln Pro Thr Gly Leu Phe Ile
 35 40 45
 Asn Gly Glu Phe Val Ala Ser Lys Gln Lys Lys Thr Phe Asp Val Ile
 50 55 60
 Asn Pro Ser Asn Glu Glu Lys Ile Thr Thr Val Tyr Lys Ala Met Glu
 65 70 75 80
 Asp Asp Val Asp Glu Ala Val Ala Ala Ala Lys Lys Ala Phe Glu Thr
 85 90 95
 Lys Trp Ser Ile Val Glu Pro Glu Val Arg Ala Lys Ala Leu Phe Asn
 100 105 110
 Leu Ala Asp Leu Val Glu Lys His Gln Glu Thr Leu Ala Ala Ile Glu
 115 120 125
 Ser Met Asp Asn Gly Lys Ser Leu Phe Cys Ala Arg Gly Asp Val Ala
 130 135 140
 Leu Val Ser Lys Tyr Leu Arg Ser Cys Gly Gly Trp Ala Asp Lys Ile
 145 150 155 160
 Tyr Gly Asn Val Ile Asp Thr Gly Lys Asn His Phe Thr Tyr Ser Ile
 165 170 175
 Lys Glu Pro Leu Gly Val Cys Gly Gln Ile Ile Pro Trp Asn Phe Pro
 180 185 190
 Leu Leu Met Trp Ser Trp Lys Ile Gly Pro Ala Leu Ala Thr Gly Asn
 195 200 205
 Thr Val Val Leu Lys Pro Ala Glu Thr Thr Pro Leu Ser Ala Leu Phe

210	215	220
Ala Ser Gln Leu Cys Gln Glu Ala Gly Ile Pro Ala Gly Val Val Asn		
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Ile Leu Pro Gly Ser Gly Arg Val Val Gly Glu Arg Leu Ser Ala His		240
	245	250
Pro Asp Val Lys Lys Ile Ala Phe Thr Gly Ser Thr Ala Thr Gly Arg		255
	260	265
His Ile Met Lys Val Ala Ala Asp Thr Val Lys Lys Val Thr Leu Glu		270
	275	280
Leu Gly Gly Lys Ser Pro Asn Ile Val Phe Ala Asp Ala Asp Leu Asp		285
290	295	300
Lys Ala Val Lys Asn Ile Ala Phe Gly Ile Phe Tyr Asn Ser Gly Glu		
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Val Cys Cys Ala Gly Ser Arg Ile Tyr Ile Gln Asp Thr Val Tyr Glu		320
	325	330
Glu Val Leu Gln Lys Leu Lys Asp Tyr Thr Glu Ser Leu Lys Val Gly		335
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Asp Pro Phe Asp Glu Glu Val Phe Gln Gly Ala Gln Thr Ser Asp Lys		350
	355	360
Gln Leu His Lys Ile Leu Asp Tyr Val Asp Val Ala Lys Ser Glu Gly		365
370	375	380
Ala Arg Leu Val Thr Gly Gly Ala Arg His Gly Ser Lys Gly Tyr Phe		
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Val Lys Pro Thr Val Phe Ala Asp Val Lys Glu Asp Met Arg Ile Val		400
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Lys Glu Glu Val Phe Gly Pro Ile Val Thr Val Ser Lys Phe Ser Thr		415
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Ala Gly Ile His Thr Asn Asp Ile Asn Lys Ala Val Asp Val Ser Lys		445
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Arg Val Lys Ala Gly Thr Val Trp Ile Asn Thr Tyr Asn Asn Phe His		
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Gln Asn Val Pro Phe Gly Gly Phe Gly Gln Ser Gly Ile Gly Arg Glu		480
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Ile Ala Ile Asp Lys Pro Ile Arg		510
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<210> 30

<211> 500

<212> PRT

<213> 酿酒酵母 (*Saccharomyces cerevisiae*)

<220>

<223> 酿酒酵母 (ALDH6) 醛脱氢酶氨基酸

序列

<400> 30

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Lys Phe Met Lys Ala Gln Asp Gly Lys Thr Tyr Pro Val Glu Asp Pro
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Ser Thr Glu Asn Thr Val Cys Glu Val Ser Ser Ala Thr Thr Glu Asp
50           55           60
Val Glu Tyr Ala Ile Glu Cys Ala Asp Arg Ala Phe His Asp Thr Glu
65           70           75           80
Trp Ala Thr Gln Asp Pro Arg Glu Arg Gly Arg Leu Leu Ser Lys Leu
85           90           95
Ala Asp Glu Leu Glu Ser Gln Ile Asp Leu Val Ser Ser Ile Glu Ala
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Leu Asp Asn Gly Lys Thr Leu Ala Leu Ala Arg Gly Asp Val Thr Ile
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Ala Ile Asn Cys Leu Arg Asp Ala Ala Ala Tyr Ala Asp Lys Val Asn
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Gly Arg Thr Ile Asn Thr Gly Asp Gly Tyr Met Asn Phe Thr Thr Leu
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Glu Pro Ile Gly Val Cys Gly Gln Ile Ile Pro Trp Asn Phe Pro Ile
165          170          175
Met Met Leu Ala Trp Lys Ile Ala Pro Ala Leu Ala Met Gly Asn Val
180          185          190
Cys Ile Leu Lys Pro Ala Ala Val Thr Pro Leu Asn Ala Leu Tyr Phe
195          200          205
Ala Ser Leu Cys Lys Lys Val Gly Ile Pro Ala Gly Val Val Asn Ile
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Val Pro Gly Pro Gly Arg Thr Val Gly Ala Ala Leu Thr Asn Asp Pro
225          230          235          240
Arg Ile Arg Lys Leu Ala Phe Thr Gly Ser Thr Glu Val Gly Lys Ser

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Leu	Gly	Gly	Lys	Ser	Ala	His	Leu	Val	Phe	Asp	Asp	Ala	Asn	Ile	Lys												
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Ile	Cys	Ser	Ser	Gly	Ser	Arg	Ile	Tyr	Val	Gln	Glu	Gly	Ile	Tyr	Asp												
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Glu	Leu	Leu	Ala	Ala	Phe	Lys	Ala	Tyr	Leu	Glu	Thr	Glu	Ile	Lys	Val												
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Gly	Asn	Pro	Phe	Asp	Lys	Ala	Asn	Phe	Gln	Gly	Ala	Ile	Thr	Asn	Arg												
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Gln	Gln	Phe	Asp	Thr	Ile	Met	Asn	Tyr	Ile	Asp	Ile	Gly	Lys	Lys	Glu												
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Gly	Ala	Lys	Ile	Leu	Thr	Gly	Gly	Glu	Lys	Val	Gly	Asp	Lys	Gly	Tyr												
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Phe	Ile	Arg	Pro	Thr	Val	Phe	Tyr	Asp	Val	Asn	Glu	Asp	Met	Arg	Ile												
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Thr	Leu	Glu	Glu	Gly	Val	Glu	Met	Ala	Asn	Ser	Ser	Glu	Phe	Gly	Leu												
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Lys	Met	Leu	Lys	Ala	Gly	Thr	Val	Trp	Ile	Asn	Thr	Tyr	Asn	Asp	Phe												
450																455						460					
Asp	Ser	Arg	Val	Pro	Phe	Gly	Gly	Val	Lys	Gln	Ser	Gly	Tyr	Gly	Arg												
465																470						475					
Glu	Met	Gly	Glu	Glu	Val	Tyr	His	Ala	Tyr	Thr	Glu	Val	Lys	Ala	Val												
485																490						495					
Arg	Ile	Lys	Leu																								
500																											