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(54)发明名称

在低温下具有改善的活性的乳糖酶

(57)摘要

本发明涉及表现出 β -半乳糖苷酶活性的新的改善的肽或二聚肽,以及特别是在低温下降低组合中乳糖含量的改善的方法。

1. 生产乳制品的方法,包括:
 - (a) 将包含浓度为至少10g/L乳糖的乳基基质与浓度为10-55mg/L、表现出 β -半乳糖苷酶活性的肽或二聚肽混合;
 - (b) 将混合物在1°C-10°C的温度下孵育足以将所述混合物中的乳糖浓度降低至小于0.2g/L的一段时间。
2. 降低乳基基质中乳糖含量的方法,包括:
 - (a) 将包含浓度为至少10g/L乳糖的乳基基质与浓度为10-55mg/L、表现出 β -半乳糖苷酶活性的肽或二聚肽混合;
 - (b) 将混合物在1°C-10°C的温度下孵育足以将所述混合物中的乳糖浓度降低至小于0.2g/L的一段时间。
3. 根据权利要求1或2所述的方法,其中,所述表现出 β -半乳糖苷酶活性的肽或二聚肽为:
 - (i) 具有选自SEQ ID NO:22、33、14、7、9、11、30和1的氨基酸序列的肽,或与任一这些序列具有超过85%的氨基酸序列同一性的肽;
 - (ii) 具有选自SEQ ID NO:22、33、14、13、19、7、9、11、26和27、30和1的氨基酸序列的肽,或与任一这些序列具有超过85%的氨基酸序列同一性的肽;
 - (iii) 具有SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33所示的氨基酸序列,或其酶促活性片段,或任一上述序列的具有不超过1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21或22个氨基酸取代、添加或缺失的氨基酸序列的肽。
4. 根据权利要求1-3中任一项所述的方法,其中,以35-52mg/L的浓度、40-52mg/L的浓度或45-52mg/L的浓度添加所述表现出 β -半乳糖苷酶活性的肽或二聚肽。
5. 根据权利要求1-4中任一项所述的方法,其中,包含乳糖的所述乳基基质是:
 - (i) 牛乳、绵羊乳、山羊乳、水牛乳、骆驼乳,或其巴氏灭菌形式、未加工形式和/或过滤形式;或
 - (ii) 通过发酵由(i)获得的发酵乳制品。
6. 根据权利要求5所述的方法,其中包含乳糖的所述乳基基质是包含浓度为约37-50g/L乳糖的牛乳,或其热处理形式、巴氏灭菌形式和/或过滤形式。
7. 根据权利要求1-5中任一项所述的方法,其中在孵育至少4小时,至少8小时,至少12小时或至少24小时之后达到小于0.2g/L乳糖的浓度。
8. 根据权利要求1-7中任一项所述的方法,其中,步骤(b)中的孵育温度在2°C-7°C的范围或在3°C-6°C的范围。
9. 根据权利要求1-8中任一项所述的方法,其中,步骤(b)中的孵育将所述混合物中的乳糖浓度降低至小于0.05g/L,小于0.02g/L或小于0.01g/L。
10. 根据权利要求1-9中任一项所述的方法,其中,在将包含所述乳基基质和所述表现出 β -半乳糖苷酶活性的肽或二聚肽的所述混合物于1°C-10°C的温度下孵育之前或之后,将所述混合物加热至至少60°C的温度,持续至少4秒。
11. 根据权利要求10所述的方法,其中在步骤(b)中在低温下孵育包含所述乳基基质和所述表现出 β -半乳糖苷酶活性的肽或二聚肽的所述混合物之前或之后,将所述混合物加热

至72°C的温度,持续约15秒,或在将所述混合物于1°C-10°C的温度下孵育之前或之后,将所述混合物加热到140°C的温度,持续约4秒。

12. 根据权利要求1或3-9中的任一项所述的生产乳制品的方法,其中,所述方法包括用乳酸菌发酵所述乳基基质的步骤。

13. 根据权利要求12所述的方法,其中在与表现出 β -半乳糖苷酶活性的肽或二聚肽孵育之前或之后进行所述发酵步骤。

14. 根据权利要求1或3-9中任一项所述的方法,其中,所述乳制品是发酵乳制品、奶酪、酸奶、黄油、乳制涂抹酱、酪乳、酸化的乳饮料、酸奶油、基于乳清的饮料、冰淇淋、炼乳、炼奶焦糖酱或调味乳饮料。

15. 表现出 β -半乳糖苷酶活性的肽或二聚肽,用于在1°C-10°C的温度下,以足以将混合物中的乳糖浓度降低至小于0.2g/L的时间段,生产乳糖含量降低的乳制品中的用途,其中所述表现出 β -半乳糖苷酶活性的肽或二聚肽为:

(i) 具有选自SEQ ID NO:22、33、14、7、9、11、30和1的氨基酸序列的肽,或与任一这些序列具有超过85%的氨基酸序列同一性的肽;

(ii) 具有选自SEQ ID NO:22、33、14、13、19、7、9、11、26和27、30和1的氨基酸序列的肽,或与任一这些序列具有超过85%的氨基酸序列同一性的肽;

(iii) 具有SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33所示的氨基酸序列,或其酶促活性片段,或任一上述序列的具有不超过1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21或22个氨基酸取代、添加或缺失的氨基酸序列的肽。

在低温下具有改善的活性的乳糖酶

技术领域

[0001] 本发明涉及使用表现出 β -半乳糖苷酶活性且在低温下具有改善的活性的新的肽或二聚肽生产乳制品的方法以及降乳制品的乳糖含量的方法。

背景技术

[0002] 为了在乳中生长,乳糖水解是乳酸菌获得作为碳源的葡萄糖和半乳糖的好方式。乳糖酶(β -半乳糖苷酶;EC 3.2.1.23)是将乳中的糖乳糖水解为单糖的酶。乳糖酶的商业用途是分解乳制品中的乳糖。乳糖不耐受人士难以消化高乳糖水平的乳制品。据估计,全世界约有70%的人消化乳糖的能力有限。因此,对不含或仅含低水平乳糖的乳制食品的需求正在增长。

[0003] 乳糖酶已从多种生物中分离出来,包括克鲁维酵母菌属(*Kluyveromyces*)和芽孢杆菌属(*Bacillus*)等微生物。克鲁维酵母菌属,特别是脆壁克鲁维酵母(*K. fragilis*)和乳酸克鲁维酵母(*K. lactis*),以及其他真菌,例如念珠菌属(*Candida*)、串酵母属(*Torula*)和球拟酵母属(*Torulopsis*)的真菌,是真菌乳糖酶的常见来源,而凝结芽孢杆菌(*B. coagulans*)和环状芽孢杆菌(*B. circulans*)是众所周知的细菌乳糖酶来源。来自这些生物的几种商业乳糖酶制剂是可用的,例如Lactozym®(可从丹麦的Novozymes获得)、HA-乳糖酶(可从丹麦的Chr. Hansen(科.汉森有限公司)获得)和Maxilact®(可从荷兰的DSM获得),所有这些制剂均来自乳酸克鲁维酵母。所有这些乳糖酶都是所谓的中性乳糖酶,其最适pH在pH6至pH8之间,最适温度在37°C左右。当此类乳糖酶用于生产例如于低乳糖酸奶时,在发酵前必须在单独步骤中进行酶处理,或者必须使用高剂量的酶,因为它们的活性会随着发酵过程中pH值的降低而降低。

[0004] 生产具有降低的乳糖的巴氏灭菌乳的典型方法包括向乳中添加乳糖酶,然后在约6°C的温度下长时间孵育(10-48h(小时),通常为24h)。因为Ha-乳糖酶和NOLA®Fit活性为每毫克酶每分钟45-70 μ mol,所以达到所需的残留乳糖水平,需要巴氏灭菌乳的酶剂量分别为55-70mg/L和45-60mg/L。Ha-乳糖酶和NOLA®Fit酶的最适温度为约37°C。在37°C下更久的孵育乳会导致微生物生长。

[0005] 而且,这些乳糖酶不适合在高温或低温下进行的乳中乳糖的水解,这在某些情况下有利于保持微生物低计数并因此确保高乳质量。此外,已知的乳糖酶不适合用于生产超热处理(UHT)乳的所需方法中,其中在UHT处理之前添加了酶。

[0006] W092/13068涉及包含由超声处理细菌或酵母的微生物细胞获得的乳糖酶活性的组合物。W02010092057和W00104276涉及冷活性 β -半乳糖苷酶。W007110619涉及具有高转半乳糖基化活性的 β -半乳糖苷酶,而W02009071539涉及具有较低的转半乳糖基化活性的 β -半乳糖苷酶。

[0007] 发明目的

[0008] 本发明实施方案的目的是,提供使用使得能够在低温下生产改善的无乳糖或低乳

糖产品的 β -半乳糖苷酶的方法。

[0009] 本发明实施方案的另一个目的是,提供使用具有以下性能的 β -半乳糖苷酶的方法,所述性能改善产品(例如无乳糖产品或低乳糖产品)中的乳糖的降低。

发明内容

[0010] 本发明人已经鉴定出具有以前未描述的性能的 β -半乳糖苷酶,所述性能使得能够生产改善的无乳糖或低乳糖产品,并且使得用于此类无乳糖或低乳糖产品的改善的生产方法成为可能。特别是,已证明这些 β -半乳糖苷酶在非常宽的温度范围和pH值范围内都非常稳定并且具有相对高的活性。它们还可以在特定温度下使用,例如在高温和在利用这些酶时通常见不到的pH值下。首先,这使得能够在不知为可能的特定pH值和温度下使用 β -半乳糖苷酶。它还使得能在几种不同的应用中使用相同的特定酶,这在工业上是非常需要的。

[0011] 在第一方面,本发明提供了用于生产乳制品的方法,包括:

[0012] (a) 将包含浓度为至少10g/L乳糖的乳基基质与表现出 β -半乳糖苷酶活性、浓度为10-55mg/L,例如20-55mg/L的肽或二聚肽混合;

[0013] (b) 将混合物在1-10°C的温度下孵育足以将混合物中的乳糖浓度降低至小于0.2g/L的一段时间。

[0014] 在相关的实施方案中,本发明提供了降低乳基基质中乳糖含量的方法,包括:

[0015] (a) 将包含浓度为至少10g/L乳糖的乳基基质与表现出 β -半乳糖苷酶活性、浓度为10-55mg/L,例如20-55mg/L的肽或二聚肽混合;

[0016] (b) 将混合物在1-10°C的温度下孵育足以将混合物中的乳糖浓度降低至小于0.2g/L的一段时间。

[0017] 上述方法可以用表现出 β -半乳糖苷酶活性的肽或二聚肽进行,所述肽或二聚肽可进一步表征为:

[0018] (i) 具有选自SEQ ID NO:22、33、14、7、9、11、30和1的氨基酸序列的肽,或与任一这些序列具有超过85%的氨基酸序列同一性的肽;

[0019] (ii) 具有选自SEQ ID NO:22、33、14、13、19、7、9、11、26和27、30和1的氨基酸序列的肽,或与任一这些序列具有超过85%的氨基酸序列同一性的肽;

[0020] (iii) 具有SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33所示的氨基酸序列,或其酶促活性片段,或任一上述序列的具有不超过1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21或22个氨基酸取代、添加或缺失的氨基酸序列的肽。

[0021] 本发明的方法之所以是有利的,是因为它们仅需要低浓度的表现出 β -半乳糖苷酶活性的肽或二聚肽,并且仍然显著降低乳糖浓度。在优选的替代方案中,以35-52mg/L的浓度、40-52mg/L的浓度或45-52mg/L的浓度添加表现出 β -半乳糖苷酶活性的肽或二聚肽

[0022] 乳基基质可以是含有乳的任何基质。一方面,上述方法使用为下述的乳基基质:

[0023] (i) 牛乳、绵羊乳、山羊乳、水牛乳、骆驼乳或其巴氏灭菌和/或过滤形式;或

[0024] (ii) 通过发酵由(i)获得的发酵乳制品。

[0025] 在特别优选的实施方案中,上述方法使用包含浓度为约37-50g/L乳糖的牛乳或其热处理形式、巴氏灭菌形式、未加工形式和/或过滤形式作为乳基基质。

[0026] 上述方法在短时间内显著降低了乳糖的浓度。在某些实施方案中,在孵育至少4小时,至少8小时,至少12小时或至少24小时后,浓度降低至小于0.2g/L乳糖的值。

[0027] 本发明方法的优点之一在于在低温下降低乳糖的浓度。例如,上述方法的步骤(b)的孵育温度可以在2°C至7°C的范围内或在3°C至6°C的范围。

[0028] 所述方法显著降低了乳糖浓度,并且优选地,步骤(b)中的孵育将混合物中的乳糖浓度降低至小于0.05g/L,小于0.02g/L或小于0.01g/L。

[0029] 具体而言,用于本发明方法中的表现出 β -半乳糖苷酶活性的肽或二聚肽不仅在低温下具有高活性,而且在高温下也具有高活性。因此,一方面,本发明提供了如上所述的方法,其中在将包含乳基基质和表现出 β -半乳糖苷酶活性的肽或二聚肽的混合物于1°C-10°C的温度下孵育之前或之后,将所述混合物加热至至少60°C的温度,持续至少四秒钟。特别是,所述方法可包括加热步骤,该步骤包括在步骤(b)中在将混合物于低温下孵育之前或之后加热至72°C的温度,持续约15秒,或在将混合物于1°C-10°C的温度下孵育之前或之后加热至140°C的温度,持续约4秒。

[0030] 在一个替代方案中,本发明的方法用于生产乳制品。这些方法还可以包括用乳酸菌发酵乳基基质的步骤。在用表现出 β -半乳糖苷酶活性的肽或二聚肽孵育之前或之后进行发酵步骤。

[0031] 所述方法特别适用于生产乳制品,例如发酵乳制品、奶酪、酸奶、黄油、乳制涂抹酱(dairy spread)、酪乳、酸化乳饮料、酸奶油(sour cream)、基于乳清的饮料、冰淇淋、炼乳、炼奶焦糖酱(dulce de leche)或调味乳饮料。

[0032] 在特别优选的实施方案中,本发明提供了生产乳或乳制品的方法,包括:

[0033] (a) 将包含浓度为至少10g/L乳糖的乳基基质和表现出 β -半乳糖苷酶活性、浓度为35-52mg/L的肽混合,其中所述肽具有选自SEQ ID NO:22、33、14、7、9、11、30和1的氨基酸序列,或与任一这些序列具有超过85%的氨基酸序列同一性;

[0034] (b) 在足以将混合物中的乳糖浓度降低至小于0.02g/L的条件下,将混合物在1°C至10°C的温度下孵育12小时。

[0035] 在另一个优选的实施方案中,本发明提供了生产乳或乳制品的方法,包括:

[0036] (a) 将包含浓度为至少10g/L乳糖的乳基基质和表现出 β -半乳糖苷酶活性、浓度为35-52mg/L的的肽混合,其中所述肽具有选自SEQ ID NO:22、33、14、7、9、11、30和1的氨基酸序列,或与任一这些序列具有超过85%的氨基酸序列同一性;

[0037] (b) 在足以将混合物中乳糖浓度降低至小于0.02g/L的条件下,将混合物在1°C至10°C的温度下孵育12小时,

[0038] 其中在将包含乳基基质和表现出 β -半乳糖苷酶活性的肽的混合物于1°C至10°C的温度下孵育之前或之后,将所述混合物加热至至少60°C的温度,持续至少4秒。

[0039] 在另一个实施方案中,本发明涉及表现出 β -半乳糖苷酶活性的肽或二聚肽,用于在1°C-10°C的温度下,以足以将混合物中的乳糖浓度降低至小于0.2g/L的时间,生产乳糖含量降低的乳制品的用途,其中所述表现出 β -半乳糖苷酶活性的肽或二聚肽为:

[0040] (i) 具有选自SEQ ID NO:22、33、14、7、9、11、30和1的氨基酸序列的肽,或与任一这些序列具有超过85%的氨基酸序列同一性的肽;

[0041] (ii) 具有选自SEQ ID NO:22、33、14、13、19、7、9、11、26和27、30和1的氨基酸序列

的肽,或与任一这些序列具有超过85%的氨基酸序列同一性的肽;

[0042] (iii) 具有SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33所示的氨基酸序列,或其酶促活性片段,或任一上述序列的具有不超过1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21或22个氨基酸取代、添加或缺失的氨基酸序列的肽。

附图说明

[0043] 图1.在pH 6.7、37℃下以乳糖为底物测定的纯化酶的比活性,描述为SUAL-1,实施例6中所讨论。在给定条件下测得的标准偏差小于6%。

[0044] 图2.在半乳糖存在的情况下,在pH 6.7、37℃测定的纯化酶的比活性,描述为SUAG,实施例7中所讨论。在给定条件下测得的标准偏差小于15%。

[0045] 图3.在pH 6.7、4℃下以乳糖为底物测定的纯化酶的比活性,描述为SUAL-2,实施例8中所讨论。在给定条件下测得的标准偏差小于5%。

[0046] 图4.在pH 6.7、43℃下以乳糖为底物测定的纯化酶的比活性,描述为SUAL-3,实施例9中所讨论。在给定条件下测得的标准偏差小于5%。

[0047] 图5.在pH 5.5、4℃下以乳糖为底物测定的纯化酶的比活性,描述为SUAL-4,实施例10中所讨论。在给定条件下测得的标准偏差小于5%。

[0048] 图6.在pH 5.5、37℃下以乳糖为底物测定的纯化酶的比活性,描述为SUAL-5,实施例11中所讨论。在给定条件下测得的标准偏差小于5%。

[0049] 图7.在pH 5.5、43℃下以乳糖为底物测定的纯化酶的比活性,描述为SUAL-6,实施例12中所讨论。在给定条件下测得的标准偏差小于5%。

[0050] 图8.在pH 4.5、4℃下以乳糖为底物测定的纯化酶的比活性,描述为SUAL-7,实施例13中所讨论。在给定条件下测得的标准偏差小于5%。

[0051] 图9.在pH 4.5、37℃下以乳糖为底物测定的纯化酶的比活性,描述为SUAL-8,实施例14中所讨论。在给定条件下测得的标准偏差小于5%。

[0052] 图10.在pH 4.5、43℃下以乳糖为底物测定的纯化酶的比活,描述为SUAL-9,实施例15中所讨论。在给定条件下测得的标准偏差小于5%。

[0053] 图11.用固定量的酶于5℃下处理24h后,使用HPLC测定的巴氏灭菌乳中残留乳糖的百分比。

[0054] 图12.用固定量的酶于25℃下处理24h后,使用HPLC测定的UHT乳中残留乳糖的百分比。

[0055] 图13.以乳糖为底物测定的纯化酶在升高的温度下的残留活性百分比。在pH 6.7、37℃下的活性视为为100%。

[0056] 图14.与乳糖酶在37℃、55℃或60℃不同温度下一起孵育后,巴氏灭菌乳中存在的残留乳糖百分比。测定中使用的LactoSens[®]试剂盒的检出限为0.01%-0.2%或0.02%-1.0%乳糖。

[0057] 图15.与浓度为0.047mg/ml的乳糖酶一起孵育后,巴氏灭菌乳中存在的残留乳糖百分比。测定中使用的LactoSens[®]试剂盒的检出限为0.01%-0.2%或0.02%-1.0%乳糖。

[0058] 图16.与乳糖酶一起孵育不同反应时间即15分钟或30分钟的巴氏灭菌乳中存在的残留乳糖百分比。测定中使用的 **LactoSens**[®]试剂盒的检出限为0.01%-0.2%或0.02%-1.0%乳糖。

[0059] 图17.与不同酶剂量即0.047mg/ml或0.024mg/ml的乳糖酶一起孵育的巴氏灭菌乳中存在的残留乳糖百分比。测定中使用的 **LactoSens**[®]试剂盒的检出限为0.01%-0.2%或0.02%-1.0%乳糖。

[0060] 图18.使用不同的剂量和不同的反应时间与乳糖酶一起孵育的巴氏灭菌乳中存在的残留乳糖百分比。测定中使用的 **LactoSens**[®]试剂盒的检出限为0.01%-0.2%或0.02%-1.0%乳糖。

[0061] 图19.在55℃下与乳糖酶一起孵育的过滤乳中存在的残留乳糖百分比。测定中使用的 **LactoSens**[®]试剂盒的检出限为0.01%-0.2%或0.02%-1.0%乳糖。

[0062] 图20.在55℃和不同酶剂量下,与乳糖酶一起孵育的过滤乳中存在的残留乳糖百分比。测定中使用的 **LactoSens**[®]试剂盒的检出限为0.01%-0.2%或0.02%-1.0%乳糖。

[0063] 图21.在55℃下与乳糖酶一起孵育不同反应时间的过滤乳中存在的残留乳糖百分比。测定中使用的 **LactoSens**[®]试剂盒的检出限为0.01%-0.2%或0.02%-1.0%乳糖。

[0064] 图22.用50mg/L剂量的Ha-乳糖酶和 **NOLA**[®]Fit在4℃下在巴氏灭菌乳中乳糖水解的动力学。将酶混合在乳中,并在4℃下储存不同的时间间隔。使用 **LactoSens**[®]测定试剂盒(Chr.Hansen,丹麦)测定残留的乳糖。测定所用的 **LactoSens**[®]试剂盒的检出限为0.01%至0.2%或0.02%-1.0%乳糖。

[0065] 图23.在添加酶12小时和24小时后测得的残留乳糖百分比。将酶在乳中混合,并在4℃下储存不同的时间间隔。使用 **LactoSens**[®]测定试剂盒(Chr.Hansen,丹麦)测定残留的乳糖。测定所用的 **LactoSens**[®]试剂盒的检出限为0.01%至0.2%或0.02%-1.0%乳糖。

[0066] 图24.用0.050mg/mL剂量的新乳糖酶在4℃下在巴氏灭菌乳中乳糖水解的动力学。将酶在乳中混合,并在4℃下储存不同的时间间隔。使用 **LactoSens**[®]测定试剂盒(Chr.Hansen,丹麦)测定残留的乳糖。使用 **NOLA**[®]Fit和Ha-乳糖酶作为对照。测定所用的 **LactoSens**[®]试剂盒的检出限为0.01%至0.2%或0.02%-1.0%乳糖。

[0067] 图25.用0.050mg/L剂量的选择的新乳糖酶在4℃下在巴氏灭菌乳中乳糖水解的动力学。图中显示了测得的残留乳糖值。将酶在乳中混合,并在4℃下储存不同的时间间隔。使用 **LactoSens**[®]测定试剂盒(Chr.Hansen,丹麦)测定残留的乳糖。使用 **NOLA**[®]Fit和Ha-乳糖酶作为对照。图中显示了测得的残留乳糖值。测定所用的 **LactoSens**[®]试剂盒的检出限为0.01%至0.2%或0.02%-1.0%乳糖。

[0068] 图26.酶剂量对乳糖水解的影响。将乳与不同剂量的酶一起孵育,混合并在4℃下储存24hr(小时)。使用 **LactoSens**[®]测定试剂盒(Chr.Hansen,丹麦)测定残留的乳糖。测定所用的 **LactoSens**[®]试剂盒的检出限为0.01%至0.2%或0.02%-1.0%乳糖。

[0069] 图27.比较不同类型乳中的酶性能。在巴氏灭菌乳和过滤乳中将乳与0.052mg/L一

起孵育,混合并在4℃下储存24小时。使用LactoSens®测定试剂盒(Chr.Hansen,丹麦)测定残留的乳糖。测定所用的LactoSens®试剂盒的检出限为0.01%至0.2%乳糖。

[0070] 图28.在pH 6.7、不同温度下测定的纯化酶的测得的比活性。比活性值定义为在给定条件下每毫克酶每分钟形成的葡萄糖的微摩尔数。在给定条件下测得的标准偏差在5-20%。

[0071] 图29.在pH 5.5、不同温度下测定的纯化酶的测得的比活性。比活性值定义为在给定条件下每毫克酶每分钟形成的葡萄糖的微摩尔数。在给定条件下测得的标准偏差为约5%。

[0072] 图30.在pH 4.5、不同温度下测定的纯化酶的测得的比活性。比活性值定义为在给定条件下每毫克酶每分钟形成的葡萄糖的微摩尔数。在给定条件下测得的标准偏差为约5%。

具体实施方式

[0073] 本发明人发现,表现出β-半乳糖苷酶活性的某些肽和二聚肽在许多不同的物理条件下令人惊讶地稳定,提供相对高的活性,超出对于这类酶通常认为是最佳的范围之外。

[0074] 因此,本发明人鉴定的这些酶在约4℃或5℃具有相对高的活性,并且因此可在生产例如鲜乳时用于乳糖水解。因此,新酶特别适合于在低温下降低诸如乳制品等乳基产品的乳糖含量。

[0075] 这些表现出β-半乳糖苷酶活性的新型改善的肽的另一个优点是,它们具有相对低的半乳糖抑制程度。这些新型酶的较低半乳糖抑制对于需要极低乳糖浓度的应用具有重要意义。

[0076] 就发酵产品的适用性而言,非常有利的是本文所述的酶在4℃-43℃相对宽的温度范围内具有高β-半乳糖苷酶酶促活性,例如在约37℃,其中发酵通常是最佳的,但是β-半乳糖苷酶的这种活性在低pH值下也是存在的,例如低至4.5,或低至4.0,或低至3.5,甚至低至pH 3。

[0077] 总而言之,本发明人发现,表现出β-半乳糖苷酶活性的某些肽在宽温度范围内具有活性,在宽pH范围内具有活性,具有通常的高水解活性而无副活性,这些肽没有或具有很小的半乳糖抑制(例如小于60%),并且它们在长期储存中稳定。

[0078] 所述β-半乳糖苷酶活性可以通过在设定条件下与乳糖一起孵育后测量释放的葡萄糖量来测定。释放的葡萄糖可以通过显色反应来检测。

[0079] 定义

[0080] 本文和本发明上下文中使用的术语“乳”应理解为通过对任何哺乳动物,例如奶牛、绵羊、山羊、水牛或骆驼挤奶而获得的乳状分泌物。

[0081] 如本文所用,术语“含有乳糖的组合物”是指含有明显可测量程度的乳糖,例如乳糖含量高于0.002%(0.002g/100ml)的任何组合物,例如任何液体。该术语涵盖乳和乳基基质。

[0082] 在本发明的上下文中,术语“乳基基质”可以是任何生乳材料和/或加工的乳材料。有用的乳基基质包括但不限于任何包含乳糖的乳或类似乳的产品的溶液/悬浮液,例如全脂乳或低脂乳、脱脂乳、酪乳、低乳糖乳、复原乳粉(reconstituted milk powder)、炼乳,乳

粉溶液、UHT乳、乳清、乳清渗透物、酸乳清、奶油、发酵乳制品的溶液,发酵乳制品例如酸奶、干酪、膳食补充剂、益生菌膳食制品。通常,术语乳基基质是指为了生产乳制品而被进一步加工的生乳材料或加工乳材料。

[0083] 本文所用的“巴氏灭菌”是指降低或消除乳基基质中活的有机体例如微生物存在的方法。优选,通过将指定温度保持指定的时间段来实现巴氏灭菌。通常通过加热来达到指定的温度。可以选择温度和持续时间,以杀死或灭活某些细菌,例如有害细菌,和/或灭活乳中的酶。随后可以进行快速冷却步骤。

[0084] 本文所用的术语“乳制品”可以是其中主要成分之一是基于乳的任何食品。通常,所述主要成分是基于乳的,并且在一些实施方案中,所述主要成分是根据本发明的方法用具有 β -半乳糖苷酶活性的酶处理的乳基基质。

[0085] 本发明的乳制品可以是例如脱脂乳、低脂乳、全脂乳、奶油、UHT乳、具有延长的保存期限的乳、发酵乳制品、奶酪、酸奶、黄油、乳制涂抹酱(dairy spread)、酪乳(butter milk)、酸化的乳饮料、酸奶油(sour cream)、基于乳清的饮料、冰淇淋、炼乳、炼奶焦糖酱(dulce de leche)或调味乳饮料。

[0086] 乳制品可以另外包含非乳成分,例如,植物成分,例如植物油、植物蛋白质和/或植物碳水化合物。乳制品还可以包含其他添加剂,例如酶、调味剂、微生物培养物(例如益生菌培养物)、盐、甜味剂、糖、酸、水果、水果制剂、果汁或本领域已知的任何其他成分,作为乳制品的成分或添加剂。

[0087] 本文所用的术语“发酵乳制品(fermented dairy product)”或“发酵乳产品(fermented milk product)”应理解为任何类型的发酵构成生产过程的一部分的任何乳制品。发酵乳制品的实例是酸奶、酪乳、鲜奶油(creme fraiche)、夸克(quark)和清爽干酪(fromage frais)等产品。发酵乳制品可以通过本领域已知的任何方法来生产或包括本领域已知的任何方法的步骤。

[0088] 本文所用的术语“发酵”是指通过微生物的作用将碳水化合物转化为醇或酸。在一些实施方案中,本发明的发酵包括将乳糖转化为乳酸。在本发明的上下文中,“微生物”可以包括能够使乳基质发酵的任何细菌或真菌。

[0089] 本文所用的术语“增加的 β -半乳糖苷酶活性”是指与参考序列相比, β -半乳糖苷酶的相对较高的比活性。

[0090] 本文所用的术语“表现出 β -半乳糖苷酶活性的肽”是指具有催化二糖乳糖水解成其组分单糖即葡萄糖和半乳糖的酶促活性的任何肽。这种肽也可以称为乳糖酶或简称为 β -半乳糖苷酶(EC:3.2.1.23)。

[0091] 在优选的实施方案中, β -半乳糖苷酶活性通过以下方式测定:将13 μ l包含已知量的纯化的乳糖酶的溶液与包含140mM乳糖的溶液在pH 6.7和37 $^{\circ}$ C下孵育10分钟,通过将温度升至95 $^{\circ}$ C持续10分钟来终止乳糖酶反应。通过将反应产物与80 μ L葡萄糖氧化酶(0.6g/L)、2,2'-连氨基双(3-乙基苯并噻唑啉-6-磺酸二铵盐)(1.0g/L ABTS)和辣根过氧化物酶(0.02g/L)的溶液在30 $^{\circ}$ C下孵育40分钟,并使用荧光光度计(FLUOphotometer)测定610nm处的吸光度,测定所形成的葡萄糖的量。吸光度与每分钟形成的葡萄糖浓度相关,并且测定的最大值(以每分钟形成的葡萄糖的微摩尔数表示)被测定为乳糖酶活性单位1(在本文中也称为UAL-1)。乳糖酶在pH6.7、37 $^{\circ}$ C下的比活性(在本文中也称为SUAL-1)定义为每毫克酶每

分钟形成的葡萄糖微摩尔数,并通过将UAL-1除以毫克表示的乳糖酶蛋白质浓度来测定。实施例6说明了进行该测定的优选替代方案的全部细节。

[0092] 虽然参考每毫克酶每分钟形成的葡萄糖的单位微摩尔值来表征 β -半乳糖苷酶的活性代表了测定活性的标准方法,但其他单位同样可以用于使用上述检测表征乳糖酶的活性。因此,一些实例通过参考每秒每 μM 酶形成的葡萄糖 μM 来表征乳糖酶活性。

[0093] 在替代的实施方案中,可以针对乳糖酶孵育使用不同的温度或不同的pH值进行测定。

[0094] 在本申请的上下文中使用的术语“肽”和“寡肽”应认为是同义的(如通常所承认的),并且当上下文需要指示通过肽基键偶联的至少两个氨基酸的链时,每个术语可以互换使用。本文使用的“多肽”一词用于含有超过十个氨基酸残基的链。本文中所有肽和多肽的式或序列按从左至右并按从氨基末端至羧基末端的方向书写。本文所用“蛋白质”是指由某些宿主生物产生的肽序列,并且可以包括翻译后修饰,例如添加的聚糖。

[0095] 本文所用的术语“氨基酸”或“氨基酸序列”是指寡肽、肽、多肽或蛋白质序列,或任一这些的片段,以及天然存在的分子或合成的分子。在本文中,“片段”是指表现出 β -半乳糖苷酶活性的肽的片段,其保留了一些酶促活性。在本文中提及“氨基酸序列”以表示天然存在的蛋白质分子的氨基酸序列时,“氨基酸序列”和类似术语并不意味着将所述氨基酸序列限制为与所述肽分子相关的完整天然氨基酸序列。

[0096] 本发明的示例性肽还包括酶的长度为至少约50、100、150、200、250、300、350、400、450、500、550、600、650、700、750、800或更多个残基的片段或酶的全长。因此,本发明的“肽片段”或“酶促活性片段”是保留至少一些功能性酶促活性的片段。通常,本发明的肽片段将会仍然含有表现出 β -半乳糖苷酶活性的肽的功能性催化结构域或其他必要活性位点。其他域可以缺失。

[0097] 通常会测量 β -半乳糖苷酶的比活性,并将其表示为每毫克所用的酶每分钟形成的葡萄糖的微摩尔。但是,该比值会根据所应用的条件例如温度和pH而变化。因此, β -半乳糖苷酶活性的值也可以称为相对于参考已知酶,例如由SEQ ID NO:34或SEQ ID NO:35定义的 β -半乳糖苷酶。

[0098] 除非另有说明,否则本文针对氨基酸所用的术语“序列同一性”是指计算为 $(n_{\text{ref}} - n_{\text{dif}}) \cdot 100 / n_{\text{ref}}$ 的序列同一性,其中 n_{dif} 是当比对时两条序列中不相同残基的总数,其中 n_{ref} 是序列之一中残基的数目。

[0099] 在一些实施方案中,序列同一性通过常规方法测定,例如Smith and Waterman, 1981, Adv. Appl. Math. 2:482, Pearson&Lipman, 1988, Proc. Natl. Acad. Sci. USA 85:2444的相似性搜索法,使用Thompson et al., 1994, Nucleic Acids Res 22:467380的CLUSTAL W算法,通过计算机执行这些算法(Genetics Computer Group的Wisconsin Genetics软件包中的GAP、BESTFIT、FASTA和TFASTA)。也可以使用BLAST算法(Altschul et al., 1990, Mol. Biol. 215:403-10),针对其软件可以通过国家生物技术信息中心www.ncbi.nlm.nih.gov/获得)。当使用任何上述算法时,将使用“窗口”长度、空位罚分等的默认参数。

[0100] 本文所述的具有具体氨基酸序列的肽可以通过氨基酸取代、添加/插入或缺失中的任何一种而不同于参考肽序列。

[0101] 本发明的一些实施方案涉及具有SEQ ID NO:1-33所示的氨基酸序列或与任一所述序列具有至少80%序列同一性的序列的肽的用途。在一些实施方案中,这种序列同一性可以为至少约81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%,例如与SEQ ID NO:1-33所示的任一参考氨基酸序列相比,具有不超过1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、15、16、17、18、19、20、21或22个氨基酸取代、添加或缺失的肽。本发明的特征还在于本发明的肽的生物活性片段。本发明的肽的生物活性片段包括这样的肽,所述肽包含与本发明的肽的氨基酸序列充分相同或由其衍生的氨基酸序列,其包含比全长蛋白质少的氨基酸,但是表现出相应全长肽的相当大一部分的生物学活性。通常,生物活性片段包含具有本发明变异蛋白质的至少一种活性的结构域或基序。本发明的肽的生物活性片段可以是例如长度为100、200、300、400、500、600、700、800、900、1000或更多个氨基酸的肽。

[0102] 如本文所用,术语“宿主细胞”包括易于用包含编码本发明肽的多核苷酸的核酸构建体或表达载体转化、转染、转导等的任何细胞类型。宿主细胞可以是衍生特定酶的细胞类型,或者其可以是对特定酶产生敏感的替代细胞类型。该术语包括野生型和减毒株。

[0103] 合适的宿主细胞可以是任何细菌,包括“乳杆菌目(Lactobacillales)”中的乳酸菌,包括乳球菌属种(Lactococcus spp.)、链球菌属种(Streptococcus spp.)、乳杆菌属种(Lactobacillus spp.)、明串珠菌属种(Leuconostoc spp.)、假明串珠菌属种(PseudoLeuconostoc spp.)、片球菌属种(Pediococcus spp.)、短杆菌属种(Brevibacterium spp.)、肠球菌属种(Enterococcus spp.)和丙酸杆菌属种(Propionibacterium spp.)。还包括属于厌氧细菌双歧杆菌即双歧杆菌属种(Bifidobacterium spp.)的产乳酸细菌,其经常单独或与乳酸菌组合用作食品培养物。该定义还包括乳酸乳球菌(Lactococcus lactis)、乳酸乳球菌乳脂亚种(Lactococcus lactis subsp.cremoris)、肠膜明串珠菌乳脂亚种(Leuconostoc mesenteroides subsp.cremoris)、肠膜假明串珠菌乳脂亚种(Pseudoleuconostoc mesenteroides subsp.cremoris)、戊糖片球菌(Pediococcus pentosaceus)、乳酸乳球菌乳酸亚种丁二酮生物变种(Lactococcus lactis subsp.lactis biovar.diacetylactis)、干酪乳杆菌干酪亚种(Lactobacillus casei subsp.casei)和副干酪乳杆菌副干酪亚种(Lactobacillus paracasei subsp.Paracasei),嗜热乳酸菌物种包括例如嗜热链球菌(Streptococcus thermophilus)、屎肠球菌(Enterococcus faecium)、德氏乳杆菌乳酸亚种(Lactobacillus delbrueckii subsp.lactis)、瑞士乳杆菌(Lactobacillus helveticus)、德氏乳杆菌保加利亚亚种(Lactobacillus delbrueckii subsp.bulgarius)和嗜酸乳杆菌(Lactobacillus acidophilus)。在该定义内的其他具体细菌包括双歧杆菌科(Bifidobacteriaceae)的细菌,例如来自双歧杆菌属(Bifidobacterium),例如来自动物双歧杆菌(bifidobacterium animalis)或长双歧杆菌(bifidobacterium longum)、青春双歧杆菌(bifidobacterium adolescentis)、两歧双歧杆菌(bifidobacterium bifidum)、短双歧杆菌(bifidobacterium breve)、链状双歧杆菌(bifidobacterium catenulatum)、婴儿双歧杆菌(bifidobacterium infantus)的菌株,或来自乳杆菌属(Lactobacillus),例如清酒乳杆菌、噬淀粉乳杆菌、德氏乳杆菌乳酸亚种和瑞士乳杆菌。

[0104] 宿主细胞的这种定义还包括以下的菌株:蘑菇属(Agaricus),例如,双孢蘑菇

(*A. bisporus*); *Ascovaginospora*; 曲霉属 (*Aspergillus*), 例如黑曲霉 (*A. niger*)、泡盛曲霉 (*A. awamori*)、臭曲霉 (*A. foetidus*)、日本曲霉 (*A. japonicus*)、米曲霉 (*A. oryzae*); 念珠菌属 (*Candida*); 毛壳属 (*Chaetomium*); *Chaetotomastia*; 网柄菌属 (*Dictyostelium*), 例如盘基网柄菌 (*D. discoideum*); 克鲁维酵母属 (*Kluveromyces*), 例如脆壁克鲁维酵母 (*K. fragilis*)、乳酸克鲁维酵母 (*K. lactis*); 毛霉属 (*Mucor*), 例如爪哇毛霉 (*M. javanicus*)、高大毛霉 (*M.ucedo*)、细孢毛霉 (*M. subtilissimus*); 脉孢菌属 (*Neurospora*), 例如粗糙脉孢菌 (*N. crassa*); 根毛霉属 (*Rhizomucor*), 例如微小根毛霉 (*R. pusillus*); 根霉属 (*Rhizopus*), 例如少根根霉 (*R. arrhizus*)、日本根霉 (*R. japonicus*)、匍枝根霉 (*R. stolonifer*); 核盘菌属 (*Sclerotinia*), 例如大豆核盘菌 (*S. libertiana*); 串酵母属 (*Torula*); 球拟酵母属 (*Torulopsis*); 毛癣菌属 (*Trichophyton*), 例如红色毛癣菌 (*Trichophyton rubrum*); 维氏核盘菌属 (*Whetzelinia*), 例如, 图维氏核盘菌 (*W. sclerotiorum*); 芽孢杆菌属 (*Bacillus*), 例如凝结芽孢杆菌 (*B. coagulans*)、环状芽孢杆菌 (*B. circulans*)、巨大芽孢杆菌 (*B. megaterium*)、休闲地芽孢杆菌 (*B. novalis*)、枯草芽孢杆菌 (*B. subtilis*)、短小芽孢杆菌 (*B. pumilus*)、嗜热脂肪芽孢杆菌 (*B. stearothermophilus*)、苏云金芽孢杆菌 (*B. thuringiensis*); 双歧杆菌属 (*Bifidobacterium*), 例如长双歧杆菌 (*B. longum*)、两歧双歧杆菌 (*B. bifidum*)、动物双歧杆菌 (*B. animalis*); 金黄杆菌属 (*Chryseobacterium*); 柠檬酸杆菌属 (*Citrobacter*), 例如弗氏柠檬酸杆菌 (*C. freundii*); 梭菌属 (*Clostridium*), 例如产气荚膜梭菌 (*C. perfringens*); 色二孢属 (*Diplodia*), 例如棉色二孢 (*D. gossypina*); 肠杆菌属 (*Enterobacter*), 例如产气肠杆菌 (*E. aerogenes*)、阴沟肠杆菌 (*E. cloacae*); 爱德华菌属 (*Edwardsiella*), 迟缓爱德华菌 (*E. tarda*); 欧文氏菌属 (*Erwinia*), 例如草生欧文氏菌 (*E. herbicola*); 埃希氏菌属 (*Escherichia*), 例如大肠杆菌 (*E. coli*); 克雷伯氏菌属 (*Klebsiella*), 例如肺炎克雷伯氏菌 (*K. pneumoniae*); *Miriococcum*; *Myrothesium*; 毛霉属 (*Mucor*); 脉孢霉属 (*Neurospora*), 例如粗糙脉孢霉 (*N. crassa*); 变形杆菌属 (*Proteus*), 例如普通变形杆菌 (*P. vulgaris*); 普罗维登斯菌属 (*Providencia*), 例如斯氏普罗维登斯菌 (*P. stuartii*); 密孔菌属 (*Pycnopus*), 例如朱红密孔菌 (*Pycnopus cinnabarinus*)、血红密孔菌 (*Pycnopus sanguineus*); 瘤胃球菌属 (*Ruminococcus*), 例如扭链瘤胃球菌 (*R. torques*); 沙门氏菌 (*Salmonella*), 例如鼠伤寒沙门氏菌 (*S. typhimurium*); 沙雷氏菌属 (*Serratia*), 例如液化沙雷氏菌 (*S. liquefaciens*)、粘质沙雷氏菌 (*S. marcescens*); 志贺氏菌属 (*Shigella*), 例如福氏志贺菌 (*S. flexneri*); 链霉菌属 (*Streptomyces*), 例如抗生素链霉菌 (*S. antibioticus*)、栗色浑圆链霉菌 (*S. castaneoglobisporus*)、紫红链霉菌 (*S. violeceoruber*); 栓菌属 (*Trametes*); 木霉属 (*Trichoderma*), 例如里氏木霉 (*T. reesei*)、绿色木霉 (*T. viride*); 耶尔森氏菌属 (*Yersinia*), 例如小肠结肠炎耶尔森氏菌 (*Y. enterocolitica*)。

[0105] 为了在低温(4-5°C)下在24小时内生产不含乳糖的巴氏灭菌乳(<0.01%残留乳糖水平), Ha-乳糖酶和NOLA®的推荐剂量为55-70mg/L(10000NLU/L)和45-60mg/L(10000BLU/L)。本发明的酶在低温下提供了非常低的残留乳糖浓度(<0.01%至0.2%)。比活性测量结果表明, 新酶的活性是Ha-乳糖酶和NOLA®Fit活性的2-5倍, 因此它们生产无乳糖乳所需的时间更少。

[0106] 下面的实施例表明,新乳糖酶比Ha-乳糖酶和NOLA®Fit更快,可在显著更短的时间内生产出无乳糖的巴氏灭菌乳。这些新酶可以减少总的处理时间。此外,使用新酶,有可能将酶剂量进一步降低25%至50%,以生产无乳糖/乳糖降低的巴氏灭菌乳。

[0107] 表1.基因编号和相应的序列识别号。

基因编号	序列身份编号	物种名称
G4	SEQ ID No 1	青春双歧杆菌
G16	SEQ ID No 2 (结构域 a) SEQ ID No 3 (结构域 b)	清酒乳杆菌
G35	SEQ ID No 4	青春双歧杆菌
G40	SEQ ID No 5 (结构域 a) SEQ ID No 6 (结构域 b)	嗜淀粉乳杆菌
G44	SEQ ID No 7	两歧双歧杆菌
G51	SEQ ID No 8	两歧双歧杆菌
G57	SEQ ID No 9	短双歧杆菌
G62	SEQ ID No 10	链状双歧杆菌
G66	SEQ ID No 11	链状双歧杆菌
G83	SEQ ID No 12	德氏乳杆菌保加利亚亚种
G84	SEQ ID No 13	德氏乳杆菌乳酸亚种
G95	SEQ ID No 14	德氏乳杆菌保加利亚亚种
G100	SEQ ID No 15	德氏乳杆菌保加利亚亚种
G104	SEQ ID No 16	德氏乳杆菌乳酸亚种
G108	SEQ ID No 17	德氏乳杆菌保加利亚亚种
G109	SEQ ID No 18	德氏乳杆菌保加利亚亚种
G118	SEQ ID No 19	德氏乳杆菌乳酸亚种
G145	SEQ ID No 20 (结构域 a) SEQ ID No 21 (结构域 b)	瑞士乳杆菌
G158	SEQ ID No 22	长双歧杆菌
G224	SEQ ID No 23 (结构域 a) SEQ ID No 24 (结构域 b)	罗伊氏乳杆菌
G256	SEQ ID No 25	德氏乳杆菌乳酸亚种
G282	SEQ ID No 26 (结构域 a) SEQ ID No 27 (结构域 b)	瑞士乳杆菌
G334	SEQ ID No 28 (结构域 a) SEQ ID No 29 (结构域 b)	卷曲乳杆菌
G335	SEQ ID No 30	嗜热链球菌
G336	SEQ ID No 31	德氏乳杆菌印度亚种
G11	SEQ ID No 32	青春双歧杆菌
G33	SEQ ID No 33	青春双歧杆菌
G600	SEQ ID No 34	两歧双歧杆菌
G500	SEQ ID No 35	乳酸克鲁维酵母

[0108]

[0109] 实施例

[0110] 一般材料和方法

[0111] 分子克隆与遗传技术

[0112] 限制性酶消化、连接、转化和其他标准分子生物学操作的技术基于文献 (Maniatis et al. "Molecular cloning:a laboratory manual,2nd edition (分子克隆:实验室手册,第二版)" Cold Spring Harbor Laboratory Press,Cold Spring Harbor,NY,1989; Sambrook and Russell "Molecular Cloning:A Laboratory Manual,3rd edition (分子克隆:实验室手册,第三版)" Cold Spring Harbor Laboratory Press,Cold Spring Harbor,

NY 2001; Miller “Experiment in molecular genetics (分子遗传学实验)” Cold Spring Harbor Laboratory Press, 1972) 中所述的方法; 或按照制造商的建议。PCR在获自 (Bio-Rad, USA) 的DNA热循环仪中进行。DNA测序由德国柏林的LGC进行。在变性条件下, 使用十二烷基硫酸钠在含有10% SDS的凝胶 (Mini-**PROTEAN**® TGX stain-free™ gel, Biorad, USA) 上通过聚丙烯酰胺凝胶电泳 (PAGE) 分析蛋白质。遵循试剂盒提供的操作方案, 使用BCA方法确定蛋白质浓度。

[0113] 细菌菌株、质粒和生长条件

[0114] 大肠杆菌 (*Escherichia coli*) 菌株TOP10 (Invitrogen) 用于克隆和分离质粒。 β -半乳糖苷酶缺陷型大肠杆菌菌株BW25113 (Δ (araD-araB) 567, Δ lacZ4787 (: :rrnB-3), λ -, rph-1, Δ (rhaD-rhaB) 568, hsdR514) (Datsenko KA, Wanner BL; 2000, Proc Natl Acad Sci U.S.A. 97:6640-6645) 与pBAD/His载体 (从Invitrogen™ Life Technologies Corporation Europe BV获得) 联合用于重组蛋白生产。

[0115] 蛋白质表达的生长培养基

[0116] 包含 (16g/L BD BBL™ Phyton™ 蛋白胨、10g/L 酵母提取物、5g/L NaCl) 的2xPY培养基用于重组蛋白生产。生长培养基补充有氨苄青霉素 (100 μ g/ml) 以维持质粒。通过向培养基中添加0.05%的阿拉伯糖来开始蛋白质生产。

[0117] 实施例1: 用于产生乳糖酶的表达载体的构建

[0118] 使用商业基因组提取试剂盒, 按照提供的方案 (DNeasy, Qiagen, 德国) 提取乳酸菌或双歧杆菌的基因组DNA。使用两个合成引物, 以纯化的基因组DNA源作为生物质, 通过PCR扩增乳糖酶基因, PCR试剂以Phusion U Hot start DNA聚合酶 (Thermo Scientific, 美国) 试剂盒提供。使用USER克隆方法 (Nour-Eldin HH, Geu-Flores F, Halkier BA, Plant Secondary Metabolism Engineering, Methods in Molecular Biology, 643; 2010) 将乳糖酶基因克隆到表达载体pBAD/His的起始密码子中, 产生表达构建体。使用USER克隆方法, 在PCR产物和目的载体上都产生了长的互补的突出端。这些突出端可以彼此退火形成稳定的杂交产物, 该杂交产物用于无需连接而转化到大肠杆菌中。为了在PCR产物中产生突出端, 在每个引物的上游区域中包括单个脱氧尿苷残基以扩增靶DNA。使用在第9位上含有尿苷 (如粗体显示) 然后是乳糖酶基因序列的正向引物 (5'-

ATTAACCAUGCGACGCAACTTCGAATGGCC-3') 和反向引物

(**ATCTTCTCUTTACCGCCTTACCACGAGCACG**) 扩增乳糖酶基因。平行地, 使用在第9位上含有单个脱氧尿苷残基 (粗体突出显示), 随后是载体DNA序列的正向 (5'-**AGAGAAGAUTTTCAGCCTGATACAGATTAAATC-3'**) 和反向引物

(5'-**ATGGTTAAUTCCTCCTGTTAGCCCAAAAAACGG-3'**) 对, 通过PCR扩增载体DNA。使用商业PCR纯化试剂盒 (Qiagen, 丹麦) 纯化PCR产物。按照提供的方案, 将纯化的PCR产物 (乳糖酶基因和载体DNA) 等摩尔量混合, 并与商业USER酶混合物 (New England Biolabs, 美国) 一起孵育。这些酶去除尿嘧啶残基以及尿苷上游的短片段, 从而在PCR产物中产生互补的突出端。这些互补的突出端彼此退火, 产生pBAD-乳糖酶表达载体。将连接混合物的等分试样转化至有化学感受态的大肠杆菌TOP 10细胞。在LB-Amp平板 (LB; Luria-

Bertani, Amp; 100 μ g/ml 氨苄青霉素) 上于37 $^{\circ}$ C选择转化体。第二天, 使用载体引物(引物1: 5'-CGGCGTCACACTTTGCTATGCC-3', 和引物2: 5'-CCGCGCTACTGCCGCCAGGC-3'), 使用过夜生长的转化体中的少量生物进行菌落PCR。将来自菌落PCR的阳性克隆在5mL LB-Amp培养基中培养, 并从细胞中分离质粒DNA。对克隆的乳糖酶基因进行测序, 以验证在基因扩增过程中没有引入其他突变。将质粒DNA转化到表达宿主大肠杆菌菌株BW25113中。

[0119] 实施例2: 乳糖酶在大肠杆菌表达宿主中的表达

[0120] 使用pBAD表达系统在大肠杆菌BW25113中产生乳糖酶。使用无菌环从Lb-Amp板中收集携带质粒DNA的新鲜转化的大肠杆菌BW25113细胞, 并用于接种5mL Lb-Amp培养基。用过夜生长的培养物(200 μ L) 在摇床(Innova[®] 42) 中的250mL烧瓶中接种50mL 2x PY培养基(含有100 μ g/mL 氨苄青霉素)。培养物在37 $^{\circ}$ C、220rpm下生长, 直到OD₆₀₀达到0.6-0.8。通过向培养基中添加0.05%阿拉伯糖来开始乳糖酶表达, 并将细胞在18 $^{\circ}$ C、180rpm下再培养16-20小时。通过离心(5000rpm, 在4 $^{\circ}$ C下10分钟) 收集细胞, 并在-20 $^{\circ}$ C下保存直至进一步使用。

[0121] 实施例3: 使用固定金属亲和色谱法进行蛋白质纯化

[0122] 将来自50mL培养物的细胞在冰上融化, 并使用10mL裂解缓冲液的混合物(BugBuster[®] (Novagen), 含有2mg/mL溶菌酶(Sigma Aldrich)、1单位全能核酸酶(Benzonase) (Sigma Aldrich) 和1X完全蛋白酶抑制剂混合物(Complete Protease inhibitor cocktail) (不含EDTA, Roche)), 通过将细胞在室温下孵育30分钟来裂解细胞。30分钟后, 通过在4 $^{\circ}$ C下16000rpm离心20分钟去除细胞碎片。将所得的上清液用0.45 μ m的孔径过滤器过滤。通过洗出乙醇和水, 用1mL浆液制备重力流Ni-Sepharose (GE Healthcare) 柱。然后用洗涤缓冲液(50mM NaH₂PO₄, pH 8.0, 含有300mM NaCl和20mM咪唑) 平衡所述柱。将无细胞的提取物上样至所述柱, 并从所述柱上洗脱未结合的蛋白质。用20mL洗涤缓冲液洗涤所述柱, 并用3.5mL洗脱缓冲液(50mM NaH₂PO₄, pH 8.0, 含有300mM NaCl和250mM咪唑) 洗脱保留的蛋白质。通过SDS-PAGE在含有10%丙烯酰胺的凝胶上分析收集的级分, 并将含有纯化的乳糖酶的凝胶合并在一起。使用预装的PD-10脱盐G-25凝胶过滤柱(GE Healthcare), 将缓冲液换成存储缓冲液(50mM KH₂PO₄缓冲液, pH 7.0, 含10mM NaCl、1mM MgCl₂)。将纯化的酶保存在4 $^{\circ}$ C下直至进一步使用。

[0123] 实施例4: 使用凝胶过滤色谱法的蛋白质纯化

[0124] 将来自50mL培养物的细胞在冰上融化, 并使用10mL裂解缓冲液的混合物(BugBuster[®] (Novagen), 含有2mg/mL溶菌酶、1单位全能核酸酶(Sigma Aldrich) 和1X完全蛋白酶抑制剂混合物(不含EDTA, Roche)), 通过将细胞在室温下(25 $^{\circ}$ C) 孵育30分钟来裂解细胞。30分钟后, 通过在4 $^{\circ}$ C下16000rpm离心20分钟除去细胞碎片。将所得的上清液用0.45 μ m的孔径过滤器过滤。遵循提供的方案, 通过用30000道尔顿过滤器(Vivaspin 20, GE Healthcare) 过滤, 浓缩澄清的无细胞提取物。用1g柱材料制备重力流Sephadex G50超细柱(Pharmacia Chemicals, 瑞典) (通过在100mL水中煮沸1小时, 冷却至室温而制备)。通过将20mL冷却的浆液施加到30mL过滤柱上来制备柱。将该柱用MilliQ水洗涤, 并用洗涤缓冲液B(50mM的NaH₂PO₄缓冲液, pH 7.0) 平衡。将500 μ L浓缩的上清液施加到所述柱上, 使上清液进入柱床。将洗涤缓冲液(50mM NaH₂PO₄缓冲液, pH 7.0) 施加到柱顶部, 并分别收集洗脱液级分。在SDS-PAGE凝胶(含有10%丙烯酰胺) 上分析收集的级分。将蛋白质级分合并在一起, 并

如前一节所述,用脱盐柱将缓冲液换成储存缓冲液(50mM KH_2PO_4 缓冲液,pH 7.0,含有10mM NaCl 、1mM MgCl_2)。将纯化的酶保存在4℃下直至进一步使用。

[0125] 实施例5:使用BCA测定的蛋白质浓度测量

[0126] 使用Pierce™ BCA蛋白测定试剂盒(Thermo Fisher Scientific,德国),按照试剂盒提供的操作方案测定纯化的乳糖酶的浓度。

[0127] 实施例6:在pH6.7、37℃下,以乳糖为底物的使用纯化酶的活性测定

[0128] 为了测量 β -半乳糖苷酶活性,将纯化的乳糖酶在缓冲液A(50mM NaH_2PO_4 缓冲液pH 6.7,含有100 μM MgSO_4)中稀释至40x。在另一个反应中,将稀释的酶与在缓冲液B中制备的乳糖溶液(在100mM pH 6.7的柠檬酸钠缓冲液中制备140mM乳糖,含有100 μM MgSO_4)一起孵育。通过在PCR管中混合13 μL 稀释的酶和37 μL 乳糖溶液来制备反应混合物。将反应混合物在DNA热循环仪中以下述孵育参数(反应时间:37℃下10分钟,酶失活:95℃下10分钟,4℃冷却)孵育。将反应混合物储存在-20℃下直至进一步使用。为了测定反应过程中形成的葡萄糖量,将10 μL 反应混合物转移至标准微量滴定板(Thermo Fischer Scientific,丹麦)的一个含有80 μL 缓冲液C(100mM NaH_2PO_4 缓冲液,pH 7.0,含有葡萄糖氧化酶;0.6g/L (Sigma Adrich)、2,2'-连氨基-双(3-乙基苯并噻唑啉-6-磺酸二铵盐);ABTS:1.0g/L (Sigma Adrich),辣根过氧化物酶;0.02g/L (Sigma Adrich))的孔中,并在30℃下孵育40分钟。40分钟后,使用FLUOStar Omega UV酶标仪(德国BMG Labtech)测定610nm处的吸光度。使用0.1-1.5之间的吸光度值进行计算,如果 $A_{610\text{ nm}}$ 值 >1.5 ,则将反应混合物用缓冲液A稀释至10倍。对于每种纯化的酶,均一式三份进行反应,三次测量的平均值用于计算。将用空pBAD/His转化的大肠杆菌细胞进行的蛋白纯化用于标准化。使用已知浓度的葡萄糖(0-2.5mM),绘制标准曲线,曲线的斜率用于计算反应期间形成的葡萄糖。用每种乳糖酶的最大吸光度值测定在37℃、pH 6.7下每分钟形成的葡萄糖的微摩尔(μmol) (例如,通过将吸光度值与使用标准曲线或校准曲线形成的葡萄糖浓度相关联),也称为乳糖酶活性单位1(或UAL-1)。在37℃、pH 6.7时的比活性(称为SUAL-1)定义为每mg酶每分钟形成的葡萄糖的微摩尔(μmol 葡萄糖/分钟/mg酶),并通过将UAL-1除以以mg的蛋白质浓度来测定。在基本上相同的条件下测定SEQ ID NO:34和SEQ ID NO:35的比活性。为了酶在鲜乳和发酵奶应用中的稳健性,非常需要在pH 6.7下的高比活性。图28描述了酶在37℃、pH 6.7下的比活性的详细结果。另外,将活性描述为每 μM 添加的酶每秒形成的葡萄糖的 μM 数。结果如图1所示。

[0129] 在pH 6.7和37℃下测定酶的比活性,且该比活性用于计算用于乳糖水解所需的大概时间,使用在pH 6.7、37℃和140mM乳糖作为底物下、基于固定酶剂量的活性单位(SUAL-1)。表2显示了有关计算乳糖水解的时间的结果:

G 编号	SUAL-1	使用以下的完全水解乳糖所需的时间		
		1 mg 酶/L (分钟)	100 mg 酶/L (秒)	47 mg 酶/L (秒)
4	118,1	1185	711	1508
11	69,2	2023	1214	2573
16	23,4	5996	3597	7626
33	130,1	1076	646	1369
40	15,8	8874	5324	11287
44	331,5	422	253	537
57	104,6	1339	803	1703
66	187,2	748	449	951
83	272,9	513	308	653
84	161,9	865	519	1100
95	288,1	486	292	618
104	90,5	1548	929	1969
108	277,9	504	302	641
118	113,8	1230	738	1565
158	254,7	550	330	699
282	58,5	2392	1435	3042
335	42,4	3298	1979	4195
500	46,9	2983	1790	3794
600	61,9	2263	1358	2879

[0130]

[0131] 表2. 在实施例6讨论的,以乳糖为底物,在37°C、pH 6.7下测定的纯化酶的比活性,描述为SUAL-1.乳糖完全水解所需的计算的时间以秒表示.在给定条件下测得的标准偏差小于6%.通过假设反应速率在整个反应期间保持不变来计算水解140mmol乳糖所需的理论时间。

[0132] 注*完全乳糖水解定义为使用基于,在pH 6.7、37°C下,以140mmol乳糖为底物的比活性单位(SUAL)的固定酶浓度,酶水解140mmol乳糖所需的时间。

[0133] 实施例7:在pH 6.7、37°C下,在半乳糖存在的情况下,使用纯化的酶进行活性测定

[0134] 将纯化的乳糖酶在缓冲液A(50mM NaH₂PO₄缓冲液,pH 6.7,含有100μM MgSO₄)中稀释至40x.在单独的反应中,将稀释的酶与缓冲液D(140mM乳糖和140mM半乳糖,在100mM、pH 6.7的柠檬酸钠缓冲液中制备,含有100μM MgSO₄)一起孵育.在PCR管中反应混合物由13μL的稀释的酶和37μL缓冲液D组成.将反应混合物在热循环仪中按照以下孵育参数(反应时间:37°C下10分钟,酶灭活:95°C下10分钟,冷却:4°C)进行孵育.将反应混合物储存在-20°C下直至进一步使用.为了测定反应过程中形成的葡萄糖量,将10μL反应混合物转移至标准微量滴定板(Thermo Fischer Scientific,丹麦)的一个含有80μL缓冲液C(100mM NaH₂PO₄缓冲液,pH 7.0,含有葡萄糖氧化酶;0.6g/L(Sigma Aldrich),2,2'-连氨基-双(3-乙基苯并噻唑啉-6-磺酸二铵盐);ABTS:1.0g/L(Sigma Aldrich),辣根过氧化物酶;0.02g/L(Sigma Aldrich))的孔中,并在30°C下孵育40分钟.40分钟后,使用FLUOStar Omega UV酶标仪(德国BMG Labtech)测定610nm处的吸光度.使用0.1-1.5之间的吸光度值进行计算,如

果A610 nm值>1.5,则将反应混合物用缓冲液A稀释至10倍。对于每种纯化的酶,反应均进行一式三份,三次测量的平均值用于计算。将用空pBAD/His转化的大肠杆菌细胞进行的蛋白质纯化用于标准化。使用已知浓度的葡萄糖(0-2.5mM),绘制标准曲线,曲线的斜率用于计算与反应过程中形成的1 μ M葡萄糖相对应的吸光度。使用每种乳糖酶的最大吸光度值测定每秒形成的葡萄糖的 μ M,描述为在pH 6.7、37 $^{\circ}$ C下,在半乳糖情况下的1个活性单位(UAG)。在半乳糖存在的情况下,在37 $^{\circ}$ C、pH 6.7时的比活性定义为每 μ M酶每秒形成的葡萄糖的 μ M数(μ M葡萄糖/秒/ μ M酶),并通过将UAG除以以 μ M的蛋白质浓度来测定,描述为SUAG。

[0135] 半乳糖对酶的抑制百分比可通过以下公式计算:

[0136] $\%抑制 = 100 * (A - B) / A$

[0137] 其中,A是如实施例6所述的在37 $^{\circ}$ C、pH 6.7下、在乳糖的情况下酶的比活性(SUAL),并且如实例7所述,B代表在37 $^{\circ}$ C、pH 6.7下,在半乳糖存在的情况下,酶的比活性(SUAG)。图2和图28描述了半乳糖抑制百分比的详细结果。较低半乳糖抑制与需要非常低的乳糖浓度的应用高度相关。

[0138] 另外,活性描述为每毫克所添加的酶每分钟形成的葡萄糖的微摩尔。结果如图28所示。

[0139] 注意:半乳糖抑制测量中相对较高的标准偏差是由于购买的半乳糖中的痕量葡萄糖杂质引起的。

[0140] 实施例8:在4 $^{\circ}$ C、pH 6.7下使用纯化的酶基于乳糖的活性测定

[0141] 将纯化的乳糖酶在缓冲液A(50mM NaH₂PO₄缓冲液pH 6.7,含有100 μ M MgSO₄)中稀释至40x。在另一个反应中,将稀释的酶与在缓冲液B中制备的乳糖溶液(在100mM、pH 6.7的柠檬酸钠缓冲液中制备的140mM乳糖,含有100 μ M MgSO₄)一起孵育。通过在PCR管中混合13 μ L稀释的纯化酶和37 μ L乳糖溶液来制备反应混合物。使用以下孵育参数(反应时间:4 $^{\circ}$ C下60分钟,酶灭活:95 $^{\circ}$ C下10分钟,储存:4 $^{\circ}$ C)在DNA热循环仪中孵育反应混合物。将反应混合物储存在-20 $^{\circ}$ C的冰箱中直至进一步使用。按照实施例6所述的方案测定反应过程中形成的葡萄糖的量。用每种乳糖酶的最大吸光度值测定每秒形成的葡萄糖的 μ M数,描述为在4 $^{\circ}$ C、pH 6.7下,在乳糖情况下的1个活性单位(UAL-2)。在4 $^{\circ}$ C、pH 6.7下的比活性定义为每 μ M酶每秒形成的葡萄糖的 μ M数(μ M葡萄糖/秒/ μ M酶),并通过将UAL-2除以以 μ M的蛋白质浓度来测定,描述为SUAL-2。对于新鲜/巴氏灭菌乳应用中的乳糖水解,在4 $^{\circ}$ C、pH 6.7下的高比活性是非常需要的。图3描述了在4 $^{\circ}$ C、pH 6.7下酶比活的详细结果。

[0142] 另外,以每毫克所添加的酶每分钟形成的葡萄糖的 μ mol数来描述活性。结果如图28中所示。

[0143] 实施例9:在43 $^{\circ}$ C、pH6.7下,使用纯化的酶以乳糖为底物的活性测定

[0144] 将纯化的乳糖酶在缓冲液A(50mM NaH₂PO₄缓冲液pH 6.7,含有100 μ M MgSO₄)中稀释至40x。在另一个反应中,将稀释的酶与在缓冲液B中制备的乳糖溶液(在100mM、pH 6.7的柠檬酸钠缓冲液中制备的140mM乳糖,含有100 μ M MgSO₄)一起孵育。通过在PCR管中混合13 μ L稀释的纯化酶和37 μ L乳糖溶液来制备反应混合物。使用以下孵育参数(反应时间:43 $^{\circ}$ C下10分钟,酶灭活:95 $^{\circ}$ C下10分钟,储存:4 $^{\circ}$ C)在DNA热循环仪中孵育反应混合物。将反应混合物储存在-20 $^{\circ}$ C的冰箱中直至进一步使用。按照实施例6所述的方案测定反应过程中形成的葡萄糖的量。用每种乳糖酶的最大吸光度值测定每秒形成的葡萄糖的 μ M数,描述为在43 $^{\circ}$ C、

pH 6.7下,在乳糖情况下的1个活性单位(UAL-3)。在43°C、pH 6.7下的比活性定义为每 μM 酶每秒形成的葡萄糖的 μM 数(μM 葡萄糖/秒/ μM 酶),并通过将UAL-3除以以 μM 的蛋白质浓度来测定,描述为SUAL-3。对于发酵乳应用中的乳糖水解,非常期望在43°C、pH 6.7下的高比活性。图4描述了在43°C、pH 6.7下酶比活性的详细结果。

[0145] 另外,以每毫克添加的酶每分钟形成的葡萄糖的微摩尔数来描述活性。结果如图28所示。

[0146] 实施例10:在4°C、pH 5.5下,使用纯化的酶在乳糖为底物上的活性测定

[0147] 将纯化的乳糖酶在缓冲液A(50mM NaH_2PO_4 缓冲液pH 6.7,含有100 μM MgSO_4)中稀释至40x。在另一个反应中,将稀释的酶与在缓冲液E中制备的乳糖溶液(在100mM、pH 5.5的柠檬酸钠缓冲液中制备的140mM乳糖,含有100 μM MgSO_4)一起孵育。通过在PCR管中混合13 μL 稀释的纯化酶和37 μL 乳糖溶液来制备反应混合物。在pH 5.5的缓冲液中制备底物溶液,并且酶溶液的pH为6.7。为了启动反应,将13 μL 酶添加到37 μL 底物溶液中。这两种缓冲液的这种混合最终使反应pH从5.5增加到5.7。

[0148] 使用以下孵育参数(反应时间:4°C下60分钟,酶灭活:95°C下10分钟,储存:4°C)在DNA热循环仪中孵育反应混合物。将反应混合物储存在-20°C的冰箱中直至进一步使用。为了测定反应过程中形成的葡萄糖的量,将10 μL 反应混合物转移至标准微量滴定板的一个含有80 μL 缓冲液C的孔中,并在30°C下孵育40分钟。40分钟后,使用FLUOStar Omega UV酶标仪(德国BMG Labtech)测定610nm处的吸光度。使用0.1-1.5之间的吸光度值进行计算,如果 $A_{610\text{ nm}}$ 值 >1.5 ,则将反应混合物用缓冲液A稀释至5x。对于每种纯化的酶,反应均按一式三份进行,三次重复测量的平均值用于计算。每种乳糖酶的最大吸光度值用于确定每秒形成的葡萄糖的 μM 数,描述为在4°C、pH 5.5下,在乳糖情况下的1个活性单位(UAL-4)。在4°C、pH 5.5下的比活性定义为每 μM 酶每秒形成的葡萄糖的 μM 数(μM 葡萄糖/秒/ μM 酶),并通过将UAL-4除以 μM 的蛋白质浓度来测定,描述为SUAL-4。在pH 5.5、4°C下的高比活性对发酵乳应用中的乳糖水解是相关的。图5描述了在4°C、pH 5.5下酶比活性的详细结果。

[0149] 另外,以每毫克添加的酶每分钟形成的葡萄糖的微摩尔数来描述活性。结果如图29所示。

[0150] 实施例11:在37°C、pH5.5下,使用纯化酶在乳糖为底物上的活性测定

[0151] 将纯化的乳糖酶在缓冲液A(50mM NaH_2PO_4 缓冲液pH 6.7,含有100 μM MgSO_4)中稀释至40x。在另一个反应中,将稀释的酶与在缓冲液E中制备的乳糖溶液(在100mM、pH 5.5的柠檬酸钠缓冲液中制备的140mM乳糖,含有100 μM MgSO_4)一起孵育。通过在PCR管中混合13 μL 稀释的纯化酶和37 μL 乳糖溶液来制备反应混合物。在pH 5.5的缓冲液中制备底物溶液,并且酶溶液的pH为6.7。为了启动反应,将13 μL 酶添加到37 μL 的底物溶液中。这两种缓冲液的这种混合最终使反应pH从5.5增加到5.7。

[0152] 使用以下孵育参数(反应时间:37°C下10分钟,酶失活:95°C下10分钟,储存:4°C)在DNA热循环仪中孵育反应混合物。将反应混合物储存在-20°C直至进一步使用。按照实施例10所述的方案测定反应期间形成的葡萄糖的量。每种乳糖酶的最大吸光度值用于测定每秒形成的葡萄糖的 μM 数,描述为在pH 5.5、37°C下,在乳糖情况下的1单位活性(UAL-5)。在pH 5.5、37°C下的比活性定义为每 μM 酶每秒形成的葡萄糖的 μM 数(μM 葡萄糖/秒/ μM 酶),并通过将UAL-5除以以 μM 的蛋白质浓度来测定,描述为SUAL-5。37°C下pH 5.5下的高比活性对

发酵乳应用中的乳糖水解和甜乳清乳糖水解是相关的。图6描述了在37°C、pH 5.5下酶的比活性的详细结果。

[0153] 另外,将活性描述为每毫克添加的酶每分钟形成的葡萄糖的微摩尔数。结果如图29所示。

[0154] 实施例12:在43°C、pH 5.5下使用纯化的酶在乳糖为底物上的活性测定

[0155] 将纯化的乳糖酶在缓冲液A (50mM NaH₂PO₄缓冲液pH 6.7,含有100μM MgSO₄)中稀释至40x。在另一个反应中,将稀释的酶与在缓冲液E中制备的乳糖溶液(在100mM、pH 5.5的柠檬酸钠缓冲液中制备的140mM乳糖,含有100μM MgSO₄)一起孵育。通过在PCR管中混合13μL稀释的纯化酶和37μL乳糖溶液来制备反应混合物。在pH 5.5的缓冲液中制备底物溶液,并且酶溶液的pH为6.7。为了启动反应,将13μL酶添加到37μL底物溶液中。这两种缓冲液的这种混合最终使反应pH从5.5增加到5.7。

[0156] 使用以下孵育参数(反应时间:43°C下10分钟,酶灭活:95°C下10分钟,储存:4°C)在DNA热循环仪中孵育反应混合物。将反应混合物储存在-20°C下直至进一步使用。按照实施例10所述的方案测定反应期间形成的葡萄糖的量。用每种乳糖酶的最大吸光度值测定每秒形成的葡萄糖的μM数,描述为在43°C、pH 5.5下,在乳糖情况下的单位活性(UAL-6)。在43°C、pH 5.5下的比活性定义为每μM酶每秒形成的葡萄糖的μM数(μM葡萄糖/秒/μM酶),并通过将UAL-6除以以μM的蛋白质浓度来测定,描述为SUAL-6。在pH 5.5、43°C下的高比活性对于发酵乳应用中的乳糖水解和甜乳清乳糖水解是相关的。图7描述了在pH 5.5、43°C下酶比活性的详细结果。

[0157] 此外,活性描述为每毫克添加的酶每分钟形成的葡萄糖微摩尔数。结果如图29所示。

[0158] 实施例13:在4°C、pH4.5下,使用纯化酶在乳糖为底物上的活性测定

[0159] 将纯化的乳糖酶在缓冲液A (50mM NaH₂PO₄缓冲液pH 6.7,含有100μM MgSO₄)中稀释至40x。在另一个反应中,将稀释的酶与在缓冲液F中制备的乳糖溶液(在100mM、pH 4.5的柠檬酸钠缓冲液中制备的140mM乳糖,含有100μM MgSO₄)一起孵育。通过在PCR管中混合13μL稀释的纯化酶和37μL乳糖溶液来制备反应混合物。在pH 4.5的缓冲液中制备底物溶液,并且酶溶液的pH为6.7。为了启动反应,将13μL酶添加到37μL底物溶液中。这两种缓冲液的这种混合最终使反应pH从4.5增加到4.7。

[0160] 使用以下孵育参数(反应时间:4°C下60分钟,酶灭活:95°C下10分钟,储存:4°C)在DNA热循环仪中孵育反应混合物。为了测定反应期间形成的葡萄糖的量,将10μL反应混合物转移至标准微量滴定板的一个含有80μL缓冲液C的孔中(如实施例6所述),并在30°C下孵育40分钟。40分钟后,使用FLUOStar Omega UV酶标仪测定在610nm处的吸光度。使用0.1-1.5的吸光度值进行计算,如果A_{610 nm}值>1.5,则将反应混合物用缓冲液A稀释至5x。对于每种纯化的酶,反应均按一式三份进行,三次重复测量的平均值用于计算。每种乳糖酶的最大吸光度值用于确定每秒形成的葡萄糖的μM数,描述为在4°C、pH 4.5下,在乳糖情况下的1单位活性(UAL-7)。在4°C、pH 4.5下的比活性定义为每μM酶每秒形成的葡萄糖的μM数(μM葡萄糖/秒/μM酶),并通过将UAL-7除以以μM的蛋白质浓度来测定,描述为SUAL-7。pH 4.5、4°C下的高比活性对于发酵乳应用中的乳糖水解是相关的。图8描述了在4°C、pH 4.5下酶比活的详细结果。

[0161] 另外,以每毫克添加的酶每分钟形成的葡萄糖微摩尔数来描述活性。结果如图30所示。

[0162] 实施例14:在37°C、pH4.5下,使用纯化的酶在乳糖为底物上的活性测定

[0163] 将纯化的乳糖酶在缓冲液A (50mM NaH₂PO₄缓冲液pH 6.7,含100μM MgSO₄)中稀释至40x。在另一个反应中,将稀释的酶与在缓冲液F中制备的乳糖溶液(在100mM、pH 4.5的柠檬酸钠缓冲液中制备的140mM乳糖,含有100μM MgSO₄)一起孵育。通过在PCR管中混合13μL稀释的纯化酶和37μL乳糖溶液来制备反应混合物。在pH 4.5的缓冲液中制备底物溶液,并且酶溶液的pH为6.7。为了启动反应,将13μL的酶添加到37μL的底物溶液中。这两种缓冲液的这种混合最终使反应pH从4.5增加到4.7。

[0164] 使用以下孵育参数(反应时间:37°C下10分钟,酶失活:在95°C下10分钟,储存:4°C)在DNA热循环仪中孵育反应混合物。将反应混合物储存在-20°C直至进一步使用。按照实施例13所述的方案测定反应期间形成的葡萄糖的量。用每种乳糖酶的最大吸光度值测定每秒形成的葡萄糖的μM数,描述为在pH 4.5、37°C下,在乳糖情况下的1单位活性(UAL-8)。在37°C、pH 4.5时的比活性定义为每μM酶每秒形成的葡萄糖的μM数(μM葡萄糖/秒/μM酶),并通过将UAL-8除以以μM的蛋白质浓度来测定,描述为SUAL-8。37°C、pH 4.5下的高比活性对发酵乳应用中的乳糖水解和酸性乳清乳糖水解是相关的。图9描述了在37°C、pH4.5下酶的比活性的详细结果。另外,将活性描述为每毫克添加的酶每分钟形成的葡萄糖的微摩尔数。结果如图30所示。

[0165] 实施例15:在43°C、pH 4.5下使用纯化的酶在乳糖为底物上的活性测定

[0166] 将纯化的乳糖酶在缓冲液A (50mM NaH₂PO₄缓冲液pH 6.7,含有100μM MgSO₄)中稀释至40x。在另一个反应中,将稀释的酶与在缓冲液F中制备的乳糖溶液(在100mM、pH 4.5的柠檬酸钠缓冲液中制备的140mM乳糖,含有100μM MgSO₄)一起孵育。通过在PCR管中混合13μL稀释的纯化酶和37μL乳糖溶液来制备反应混合物。在pH 4.5的缓冲液中制备底物溶液,并且酶溶液的pH为6.7。为了启动反应,将13μL酶添加到37μL底物溶液中。这两种缓冲液的这种混合最终使反应pH从4.5增加到4.7。使用以下孵育参数(反应时间:43°C下10分钟,酶灭活:95°C下10分钟,储存:4°C)在DNA热循环仪中孵育反应混合物。将反应混合物储存在-20°C下直至进一步使用。按照实施例13所述的方案测定反应期间形成的葡萄糖的量。用每种乳糖酶的最大吸光度值测定每秒形成的葡萄糖的μM数,描述为在43°C、pH 4.5下,在乳糖情况下的1单位活性(UAL-9)。在43°C、pH 4.5下的比活性定义为每μM酶每秒形成的葡萄糖的μM数(μM葡萄糖/秒/μM酶),并通过将UAL-9除以以μM的蛋白质浓度来测定,描述为SUAL-9。在43°C、pH 4.5下的高比活对于发酵乳应用中的乳糖水解和酸性乳清乳糖水解是相关的。图10描述了在43°C、pH为4.5下酶比活性的详细结果。

[0167] 此外,以每毫克添加的酶每分钟形成的葡萄糖的微摩尔数描述活性。结果如图30所示。

[0168] 实施例16:以BLU为单位的活性测定

[0169] 在缓冲液G (50mM NaH₂PO₄ pH 7.0缓冲液,含有100μM MgSO₄、0.045%Brij,Sigma Aldrich)中,将商购可获得的NOLA®Fit酶(Chr-Hansen,丹麦)稀释为0.5BLU/mL至2.5BLU/mL。将稀释的酶与在缓冲液H中制备的乳糖溶液(在100mM、pH 6.7的柠檬酸钠缓冲液中制备的105mM乳糖,含有100μM MgSO₄)一起孵育。通过在PCR管中混合13μL稀释的纯化

酶和37 μ L乳糖溶液来制备反应混合物。使用以下孵育参数(反应时间:37 $^{\circ}$ C下10分钟,酶灭活:95 $^{\circ}$ C下10分钟,储存:4 $^{\circ}$ C)在DNA热循环仪中孵育反应混合物。通过将10 μ L反应混合物转移至标准微量滴定板的含有80 μ L缓冲液C的单个孔中,并在30 $^{\circ}$ C下孵育40分钟,来测定葡萄糖转化量。40分钟后,使用FLUOStar Omega UV酶标仪(德国BMG Labtech)测定610nm处的吸光度。使用测得的吸光度值绘制针对BLU/mL的标准曲线。曲线的最大斜率用于测定新酶以BLU/mL为单位的活性。

[0170] 实施例17:使用乳糖作为底物以BLU/mL进行新乳糖酶的活性测定

[0171] 将纯化的乳糖酶在缓冲液A(50mM NaH₂PO₄缓冲液pH 6.7,含有100 μ M MgSO₄)中稀释至50x。在另一个反应中,将稀释的酶与在缓冲液H中制备的乳糖溶液(在100mM、pH 6.7的柠檬酸钠缓冲液中制备的105mM乳糖,含有100 μ M MgSO₄)一起孵育。通过在PCR管中混合13 μ L稀释的纯化酶和37 μ L乳糖溶液来制备反应混合物。使用以下孵育参数(反应时间:37 $^{\circ}$ C下10分钟,酶灭活:95 $^{\circ}$ C下10分钟,储存:4 $^{\circ}$ C)在DNA热循环仪中孵育反应混合物。反应后,将10 μ L反应混合物转移至标准微量滴定板的一个含有80 μ L缓冲液C的孔中(如实施例6所述),并在30 $^{\circ}$ C下孵育40分钟。40分钟后,使用FLUOStar Omega UV酶标仪在610nm处测定吸光度。使用0.1-1.5之间的吸光度值进行计算,如果A_{610nm}值>1.5,则将反应混合物用缓冲液A稀释至5x。最大吸光度值用于利用实施例16所述的标准曲线计算以BLU/mL的酶活性。

[0172] 实施例18:低温下鲜乳中残留乳糖百分比的测量

[0173] 在10mL玻璃管中,将2mL商业巴氏灭菌乳(1.5%脂肪的巴氏灭菌乳,Arla Food)与10-125 μ L如实施例17所测定的酶(相当于10BLU/mL)混合。将样品在恒定条件下于4 $^{\circ}$ C孵育24小时。孵育后,通过在95 $^{\circ}$ C下热灭活7分钟来终止反应,然后在-20 $^{\circ}$ C储存直至进一步使用。使用HPLC测定法分析乳中残留乳糖的量。将分析样品用1.8mL蛋白质沉淀溶液(0.083M PCA和2mM Na-EDTA)和2mL MQW处理,然后在4 $^{\circ}$ C下以2800rpm离心30分钟。使用Janus稀释机器人(PerkinElmer,沃尔瑟姆,马萨诸塞州,美国)将上清液的等分试样总共稀释200倍。稀释后的样品在Dionex ICS-5000系统(Thermo Fischer Scientific,沃尔瑟姆(马萨诸塞州),美国)上使用4x 250mm CarboPac SA20分析柱(Thermo Fischer Scientific,沃尔瑟姆,马萨诸塞州,美国)和脉冲安培检测器进行分析。将检测器设置为选择性检测碳水化合物的简单的三步电势波形。洗脱液设定为1mM KOH,并通过捕集柱(CR-TC, Thermo Fischer Scientific,沃尔瑟姆(马萨诸塞州),美国)连续再生。洗脱液的流速为1.2mL/min,分析时间为每次进样10分钟。使用三点外部校准曲线对每个样品中的乳糖进行定量,该校准曲线是通过将已知量的乳糖一水合物(Sigma-Aldrich,美国密苏里州圣路易斯)添加到MQW中制得的。根据色谱峰高度计算浓度。鲜乳中测量的残留乳糖百分比如图11所示。

[0174] 实施例19:室温下UHT乳中的活性测定

[0175] 在10mL玻璃管中将2mL UHT乳(1.5%脂肪UHT乳,Arla Food)与2-25 μ L如实施例17中所测定的酶(相当于2BLU/mL)混合。将样品在恒定条件下于25 $^{\circ}$ C孵育24小时。孵育后,通过在95 $^{\circ}$ C下热灭活7分钟来终止反应,然后在-20 $^{\circ}$ C储存直至进一步使用。按照如实施例18所述的方案,使用HPLC分析UHT乳中残留乳糖的量。图12列出了水解后鲜乳中残留乳糖的百分比。

[0176] 实施例20:缓冲液中高温下的酶性能

[0177] 将纯化的酶在缓冲液A(50mM NaH₂PO₄缓冲液pH 6.7,含有100 μ M MgSO₄)中稀释至

5BLU/mL。在另一个反应中,将13 μ L稀释酶在DNA热循环仪中与乳糖溶液(在100mM、pH 6.7的柠檬酸钠缓冲液中制备的105mM乳糖,含有100 μ M MgSO₄)一起孵育。通过在PCR管中混合13 μ L酶和37 μ L乳糖溶液来制备反应混合物。使用以下孵育参数(反应时间:37 $^{\circ}$ C下10分钟,酶灭活:95 $^{\circ}$ C下10分钟,储存:4 $^{\circ}$ C)在DNA热循环仪中孵育反应混合物。反应后,将10 μ L反应混合物转移至标准微量滴定板的一个含有80 μ L缓冲液C的孔中(如实施例6所述),并在30 $^{\circ}$ C下孵育40分钟。40分钟后,使用FLUOStar Omega UV酶标仪在610nm处测定吸光度。使用0.1-1.5之间的吸光度值进行计算,如果A_{610 nm}值>1.5,则将反应混合物用缓冲液A稀释至5x。测得的吸光度称为Abs_{37 $^{\circ}$ C},并视为计算的参考值。

[0178] 为了测量热处理对酶活性的影响,在单独的反应中,使用以下孵育参数(72 $^{\circ}$ C下15秒,或74 $^{\circ}$ C下15秒,或76 $^{\circ}$ C下6秒,或78 $^{\circ}$ C下6秒,或80 $^{\circ}$ C下4秒,或85 $^{\circ}$ C下5秒,或90 $^{\circ}$ C下5秒,或95 $^{\circ}$ C下5秒,然后在4 $^{\circ}$ C下储存)在DNA热循环仪中孵育13 μ L稀释的酶(5BLU/mL)。如上所述,通过与乳糖溶液(在100mM、pH 6.7的柠檬酸钠缓冲液中制备的105mM乳糖,含有100 μ M MgSO₄)一起孵育来测定热处理的酶的活性。在不同温度下(例如在72 $^{\circ}$ C、74 $^{\circ}$ C、76 $^{\circ}$ C、78 $^{\circ}$ C、80 $^{\circ}$ C、85 $^{\circ}$ C、90 $^{\circ}$ C或95 $^{\circ}$ C下)测得的吸光度称为Abs_{72 $^{\circ}$ C}、Abs_{74 $^{\circ}$ C}、Abs_{76 $^{\circ}$ C}、Abs_{78 $^{\circ}$ C}、Abs_{80 $^{\circ}$ C}、Abs_{85 $^{\circ}$ C}、Abs_{90 $^{\circ}$ C}、Abs_{95 $^{\circ}$ C}。

[0179] 使用以下公式测定高温下的残留活性百分比:

[0180] $\% \text{残留活性} = (\text{Abs}_{72^{\circ}\text{C}} / \text{Abs}_{37^{\circ}\text{C}}) * 100$

[0181] 图13描述了不同酶在不同温度下的残留活性百分比。

[0182] 实施例21:高热处理后的残留乳糖百分比

[0183] 通过在乳中孵育固定量的酶,然后进行热处理,测定热处理对巴氏灭菌乳中酶性能的影响。在单独的反应中,在PCR管中将50 μ L的巴氏灭菌乳与10BLU/mL的纯化酶(如实施例17所测定)混合。将乳样品在72 $^{\circ}$ C下孵育15秒,或在76 $^{\circ}$ C下孵育10秒,或在85 $^{\circ}$ C下孵育5秒,和在90 $^{\circ}$ C下孵育5秒,然后在5 $^{\circ}$ C孵育24小时。在5 $^{\circ}$ C下24h(小时)后,通过在95 $^{\circ}$ C下将反应加热7分钟来终止反应,然后在-20 $^{\circ}$ C下储存。按照提供的方案,使用LactoSens[®]测定试剂盒(Chr. Hansen, 丹麦),测量残留乳糖。在不同温度下以g/L测定测量的残留乳糖。

LactoSens[®]试剂盒的检出限为0.2g/L至10g/L。结果如表3所述:

[0184] 表3:用固定量的纯化酶处理,然后在不同温度下孵育(72 $^{\circ}$ C下15秒,76 $^{\circ}$ C下10秒,85 $^{\circ}$ C下5秒和90 $^{\circ}$ C下5秒,然后4 $^{\circ}$ C下孵育24h)的巴氏灭菌乳中残留乳糖的百分比,其使用LactoSens[®]测定试剂盒测定。LactoSens[®]试剂盒的检出限为0.2g/L至10g/L乳糖。这里ND;未测定到。

G 编号	在以下的残留乳糖				
	4°C (g/L)	72°C (g/L)	76°C (g/L)	85°C (g/L)	90°C (g/L)
G4	<0.2	> 10.0	ND	ND	ND
G11	<0.2	> 10.0	ND	ND	ND
G16	<0.2	> 10.0	ND	ND	ND
G33	<0.2	4.7	ND	ND	ND
G35	<0.2	> 10.0	> 10.0	ND	ND
G40	<0.2	<0.2	<0.2	> 10.0	ND
G44	0.9	> 10.0	ND	ND	ND
G57	<0.2	> 10.0	ND	ND	ND
G62	8.4	> 10.0	> 10.0	> 10.0	ND
G66	0.35	> 10.0	ND	ND	ND
G83	0.3	2.1	6.0	> 10.0	ND
G84	0.25	0.65	0.5	7.6	>10
G95	0.3	6.0	8.6	>10	ND
G100	0.4	2.4	2.6	> 10.0	ND
G104	0.35	0.45	0.5	0.45	>10
G108	0.35	1.3	1.55	ND	ND
G109	0.35	1.45	3.4	> 10.0	ND
G118	0.45	0.95	0.8	> 10.0	>10
G158	<0.2	3.9	> 10.0	ND	ND
G256	0.3	1.0	0.75	3.4	>10
G282	<0.2	<0.2	< 0.2	<0.2	>10
G335	<0.2	0.35	8.0	> 10.0	ND
G600	<0.2	> 10.0	> 10.0	> 10.0	ND
G500	<0.2	> 10.0	ND	ND	ND

[0185] [0186] 实施例22:在不同温度下孵育的巴氏灭菌乳中残留乳糖百分比

[0187] 在1.5mL Eppendorf管中,将1mL商业巴氏灭菌乳(1.5%脂肪乳,含有4.7%的乳糖,丹麦Arla Foods)与0.047mg/mL的酶混合。将酶在乳中通过轻轻涡旋或吹打混合。将50μL含有酶的乳转移到PCR管中。对于每种酶,反应以2x50μL反应体积进行。将反应混合物在DNA热循环仪中以下述孵育参数(反应温度和时间:37°C30分钟,或55°C30分钟,或60°C30分钟,酶灭活温度和时间:95°C10分钟,储存温度:4°C)进行孵育。在添加酶的过程中,移液和混合乳样品在冰水混合物中进行,以使温度对酶性能的影响最小化。反应后,将乳样品直接用于残留乳糖的测量,或保存在-20°C下直至进一步使用。使用LactoSens®测定试剂盒(Chr.Hansen,丹麦)按照试剂盒提供的方案,分析乳中的残留乳糖。巴氏灭菌乳中测得的残留乳糖百分比如图14所示。

[0188] 为了测试这些新乳糖酶的乳糖水解潜力,我们每毫升巴氏灭菌乳孵育了0.047mg酶,并在37°C、55°C和60°C下孵育30分钟。孵育30分钟后,通过在95°C下加热使酶失活。使用LactoSens®测定试剂盒(Chr.Hansen,丹麦)测定残留的乳糖。在最适温度(37°C)下,Ha-

乳糖酶和NOLA®fit均显示出高残留乳糖 (>1%的残留乳糖), 这表明在给定的时间范围内, 这些酶的活性较低且不会产生无乳糖的巴氏灭菌乳。而且, 在55°C和60°C下测量到相似的残留乳糖水平。相反, G33、G44、G95和G158酶在37°C下显示<0.1%的残留乳糖, 图15。由于它们在升高的温度(55°C或60°C)下的高活性, 因此在孵育30分钟后所述新酶表现出<0.01%的残留乳糖。这表明通过使用当前的酶剂量, 有可能在不到30分钟的时间内生产出基本不含乳糖的巴氏灭菌的且过滤的乳。相比像巴氏灭菌乳, 过滤的乳更像生乳。短时间内在升高的温度(55°C-60°C)下的乳糖水解可降低微生物生长的机会, 而不会影响乳的质量。

[0189] 实施例23: 在不同时间段内孵育的巴氏灭菌乳中的残留乳糖百分比

[0190] 在1.5mL Eppendorf管中, 将1mL商业巴氏灭菌乳(1.5%脂肪乳, 含有4.7%的乳糖, 丹麦Arla Foods)与0.047mg/mL的酶混合。将酶在乳中通过轻轻涡旋或吹打混合。将50μL含有酶的乳转移到PCR管中。对于每种酶, 反应以2x50μL的反应体积进行。将反应混合物在DNA热循环仪中按照以下孵育参数(反应温度和时间: 55°C 15分钟或55°C 30分钟, 酶灭活温度和时间: 95°C 10分钟, 储存温度: 4°C)进行孵育。在添加酶的过程中, 移液和混合乳样品在冰水混合物上进行, 以使温度和时间的影响最小化。反应后, 乳样品要么直接用于残留乳糖的测量, 要么储存在-20°C下直至进一步使用。如实施例22所述, 使用LactoSens®测定试剂盒(Chr. Hansen, 丹麦)分析乳中的残留乳糖。巴氏灭菌乳中残留乳糖的测量百分比如图16所示。

[0191] 实施例24: 以不同酶剂量孵育的巴氏灭菌乳中的残留乳糖百分比

[0192] 在1.5mL Eppendorf管中, 将1mL商业巴氏灭菌乳(1.5%脂肪乳, 含有4.7%的乳糖, 丹麦Arla Foods)与不同剂量的酶(0.024mg/mL或0.047mg/mL)混合。将酶在乳中通过轻轻涡旋或吹打混合。将50μL含有酶的乳转移到PCR管中。对于每种酶, 反应以2x50μL的反应体积进行。将反应混合物在DNA热循环仪中按照以下孵育参数(反应温度和时间: 55°C 30分钟, 酶灭活温度和时间: 95°C 10分钟, 储存温度: 4°C)进行孵育。反应后, 样品或直接用于残留乳糖的测量, 或储存在-20°C下直至进一步使用。按照与实施例22所述相同的方案分析乳中的残留乳糖。巴氏灭菌乳中的测量的残留乳糖百分比如图17所示。

[0193] 实施例25: 用不同的酶剂量和在不同的反应时间段跨度内孵育的巴氏灭菌乳中的残留乳糖百分比

[0194] 在1.5mL Eppendorf管中, 将1mL商业巴氏灭菌乳(1.5%脂肪乳, 含有4.7%的乳糖, 丹麦Arla Foods)与不同剂量的酶(0.024或0.047mg/mL)混合。将酶在乳中通过轻轻涡旋或吹打混合。将50μL含有酶的乳转移到PCR管中。对于每种酶, 反应以2x50μL的反应体积进行。将反应混合物在DNA热循环仪中按照以下孵育参数(反应温度和时间: 55°C 15分钟或55°C 30分钟, 酶灭活温度和时间: 95°C 10分钟, 储存温度: 4°C)进行孵育。在添加酶的过程中, 移液和混合乳样品在冰水混合物上进行, 以使温度和时间的影响最小化。反应后, 样品或直接用于残留乳糖的测量, 或储存在-20°C下直至进一步使用。使用实施例22所述的方案测定残留乳糖。巴氏灭菌乳中的测量的残留乳糖百分比如图18所示。

[0195] 实施例26: 过滤乳中残留乳糖的百分比

[0196] 在1.5mL Eppendorf管中, 将1mL商业微滤半脱脂乳(1.5%脂肪乳, 含有4.8%的乳糖, Marguerite, 法国)与0.047mg/mL的酶混合。将酶在乳中通过轻轻涡旋或吹打混合。将50μL含有酶的乳转移到PCR管中。对于每种酶, 反应以2x50μL的反应体积进行。将反应混合物

在DNA热循环仪中按照以下孵育参数(反应温度和时间:55℃30分钟,酶灭活温度和时间:95℃10分钟,储存温度:4℃)进行孵育。在添加酶的过程中,移液和混合乳样品在冰水混合物上进行,以使温度和时间的影响最小化。反应后,样品或直接用于残留乳糖的测量,或储存在-20℃下直至进一步使用。使用LactoSens®测定试剂盒(Chr.Hansen,丹麦),按照试剂盒提供的方案,分析乳中残留乳糖的量。过滤乳中的测量的残留乳糖百分比如图19所示。

[0197] 这表明通过使用当前的酶剂量,有可能在不到30分钟的时间内生产不含乳糖的过滤乳(与像巴氏灭菌乳相比,过滤乳更像生乳)。短时间内在升高的温度(55℃-60℃)下的乳糖水解减少微生物生长的机会,而不会影响乳的质量。

[0198] 实施例27:用不同剂量的酶孵育的过滤乳中的残留乳糖百分比

[0199] 在1.5mL Eppendorf管中,将1mL商业微滤半脱脂乳(1.5%脂肪乳,含有4.8%的乳糖,Marguerite,法国)与不同剂量的酶(0.055mg/mL,0.55μM,或0.11mg/mL,0.11μM)混合。将酶在乳中通过轻轻涡旋或吹打混合。将50μL含有酶的乳转移到PCR管中。对于每种酶,反应以2x50μL的反应体积进行。将反应混合物在DNA热循环仪中按照以下孵育参数(反应温度和时间:55℃5分钟,酶灭活温度和时间:95℃10分钟,储存温度:4℃)进行孵育。在添加酶的过程中,移液和混合乳样品在冰水混合物上进行,以使温度和时间的影响最小化。反应后,样品或直接用于残留乳糖的测量,或储存在-20℃下直至进一步使用。按照实施例22所述的方案分析乳中的残留乳糖。过滤乳中的测量的残留乳糖百分比如图20所示。

[0200] 实施例28:在不同时间跨度内孵育的过滤乳中的残留乳糖百分比

[0201] 在1.5mL Eppendorf管中,将1mL商业微滤半脱脂乳(1.5%脂肪乳,含有4.8%的乳糖,Marguerite,法国)与0.11mg/mL(1μM)的酶混合。将酶在乳中通过轻轻涡旋或吹打混合。将50μL含有酶的乳转移到PCR管中。对于每种酶,反应以2x50μL的反应体积进行。将反应混合物在DNA热循环仪中按照以下孵育参数(反应温度和时间:55℃5分钟或55℃6分钟或55℃7分钟;酶灭活温度和时间:95℃10分钟,储存温度:4℃)进行孵育。在添加酶的过程中,移液和混合乳样品在冰水混合物上进行,以使温度和时间的影响最小化。反应后,样品或直接用于残留乳糖的测量,或储存在-20℃直至进一步使用。使用LactoSens®测定试剂盒(Chr.Hansen,丹麦),按照试剂盒提供的方案,分析乳中残留乳糖的量。过滤乳中的测量的残留乳糖的百分比如图21所示。这表明通过使用当前的酶剂量,有可能在不到5-30分钟的时间内生产不含乳糖的巴氏灭菌过滤乳(与像巴氏灭菌乳相比,过滤乳更像生乳)。短时间内在升高的温度(55℃-60℃)下的乳糖水解减少微生物生长的机会,而不会影响乳的质量。

[0202] 实施例29:4-5℃下的酶活性

[0203] 为了分析新酶在巴氏灭菌乳中水解乳糖的动力学,向每毫升商业巴氏灭菌乳(含4.7%乳糖的1.5%脂肪乳,Arla Foods,丹麦)中添加0.05mg酶。通过轻轻涡旋将酶充分混合,然后转移到PCR试管中,每管10x100μL。将反应混合物在4℃下孵育,并在固定间隔后取出样品。通过在PCR仪中于95℃加热10分钟来终止反应。将样品冷却至室温,并使用LactoSens®测定试剂盒(Chr.Hansen,丹麦)测量残留的乳糖。将残留乳糖的测量值对反应时间作图。

[0204] 在4-5℃下,已知的商业产品即NOLA®Fit(G600)和Ha-乳糖酶™(G500)分别需要8-12小时和18-24小时才能将牛乳中残留的乳糖浓度分别降低到小于<0.1%和<0.01%

(如图22和23所示)。

[0205] 在这些条件下,本发明的乳糖酶明显更具活性。例如,G95(活性最高的酶)实现乳糖残留浓度<0.1%的水平(4小时)。G158和G33能够在5-6小时将乳糖残留浓度降低至<0.1%的水平,并在8-12小时将乳糖浓度降低至<0.01%的水平。孵育12小时后,几种乳糖酶显示出比对照酶更低的残留乳糖(如图24和25所示)。这些结果表明,新乳糖酶比Ha-乳糖酶™和NOLA®Fit更快,并在显著更短的时间内生产出不含乳糖的巴氏灭菌乳。这些新酶可将总处理时间减少50%。另外,新酶提供了将酶剂量进一步降低25-50%,以生产无乳糖/乳糖降低的巴氏灭菌乳的可能性(如图26所示)。

[0206] 因此,这些结果表明,新乳糖酶以50mg/L的酶剂量可以在显著更短的时间(8-12小时)内生产无乳糖巴氏灭菌乳。而且,取决于所需的乳糖水平,可以将酶剂量降低25-50%。

[0207] 实施例30:4-5°C下不同类型的乳中的酶活性

[0208] 为了比较不同类型的乳中的酶活性,使用浓度为0.052mg/L的乳糖酶孵育巴氏灭菌过滤乳。将样品混合并在4°C下储存24小时。

[0209] 使用LactoSens®测定试剂盒(Chr.Hansen,丹麦)测定残留的乳糖含量,如图27所示,图27表明许多新的乳糖酶在4°C在消化巴氏灭菌和过滤乳中的乳糖中具有很高的活性。

序列表

<110> 科·汉森有限公司

<120> 具有改善的性质的乳糖酶

<130> P 102838

<160> 41

<170> PatentIn version 3.5

<210> 1

<211> 1049

<212> PRT

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

<400> 1

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

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210	215	220
His Gly Leu Phe Arg Thr Val Glu Leu Ala Ala Gln Pro His Thr His		
225	230	235
Val Glu Thr Val Gln Leu Glu Ala Asp Tyr Thr Ala Ala Asp Thr Ala		
	245	250
Gly Thr Ala Asp Thr Ala Glu Leu Asn Ala Ala Leu Thr Leu Arg Asn		
	260	265
Ser Ala Asp Ala Met Thr Ile Glu Ser Thr Leu Arg Asp Gly Asp Gly		
	275	280
Asn Val Val Trp Glu Ser Thr Gln Ala Cys Asn Gly Glu Ile Ala Leu		
290	295	300
Asn Ser Gly Lys Met Thr Asn Ile Ala Pro Trp Ser Ala Glu Ser Pro		
305	310	315
Thr Leu Tyr Thr Leu Thr Val Arg Val Val Gly His Asp Gly Ala Ile		
	325	330
Ile Glu Thr Val Thr Gln Lys Ile Gly Phe Arg Thr Phe Arg Ile Glu		
	340	345
Asn Gly Ile Met Thr Leu Asn Gly Lys Arg Ile Val Phe Lys Gly Ala		
355	360	365
Asp Arg His Glu Phe Asp Ala Lys Arg Gly Arg Ala Ile Thr Arg Glu		
370	375	380
Asp Met Leu Ser Asp Val Val Phe Cys Lys Arg His Asn Ile Asn Ala		
385	390	395
Ile Arg Thr Ser His Tyr Pro Asn Gln Glu Tyr Trp Tyr Asp Leu Cys		
	405	410
Asp Glu Tyr Gly Leu Tyr Leu Ile Asp Glu Thr Asn Met Glu Thr His		
	420	425
Gly Thr Trp Val Ala Asn Asn Val Glu Arg Pro Glu Asp Gly Ile Pro		
435	440	445
Gly Ser Arg Pro Glu Trp Glu Gly Ala Cys Val Asp Arg Ile Asn Ser		
450	455	460
Met Met Arg Arg Asp Tyr Asn His Pro Ser Val Leu Ile Trp Ser Leu		
465	470	475
Gly Asn Glu Ser Ser Ala Gly Glu Val Phe Arg Ala Met Tyr Arg His		
	485	490
Ala His Thr Ile Asp Pro Asn Arg Pro Val His Tyr Glu Gly Ser Val		
	500	505
His Met Arg Glu Phe Glu Asp Val Thr Asp Ile Glu Ser Arg Met Tyr		
515	520	525

Ala His Ala Asp Glu Ile Glu Arg Tyr Leu Asn Asp Gly Ser Pro Ala
 530 535 540
 His Thr Asp Gly Pro Lys Lys Pro Tyr Ile Ser Cys Glu Tyr Met His
 545 550 555 560
 Ala Met Gly Asn Ser Cys Gly Asn Met Asp Glu Tyr Thr Ala Leu Glu
 565 570 575
 Arg Tyr Pro Met Tyr Gln Gly Gly Phe Ile Trp Asp Phe Ile Asp Gln
 580 585 590
 Ala Ile Glu Thr Lys Leu Pro Asp Gly Thr Thr Arg Met Cys Tyr Gly
 595 600 605
 Gly Asp Phe Gly Asp Arg Pro Ser Asp Tyr Glu Phe Ser Gly Asp Gly
 610 615 620
 Leu Leu Phe Ala Asp Arg Thr Pro Ser Pro Lys Ala Gln Glu Val Lys
 625 630 635 640
 Gln Leu Tyr Ala Asn Val Lys Ile Val Val Ser Val Asp Glu Ala Arg
 645 650 655
 Ile Thr Asn Asp Asn Leu Phe Val Ser Thr Gly Asp Tyr Arg Phe Val
 660 665 670
 Leu Arg Ile Leu Ala Asp Gly Lys Pro Val Trp Ser Thr Thr Arg Arg
 675 680 685
 Phe Asp Val Ala Ala Gly Glu Ser Ala Ser Phe Glu Val Asp Trp Pro
 690 695 700
 Val Asp Asp Tyr Arg Ser Asn Ala Glu Glu Leu Val Leu Glu Val Ser
 705 710 715 720
 Gln Gln Leu Gly Asn Ala Cys Asp Trp Ala Pro Ala Gly Tyr Glu Leu
 725 730 735
 Ala Phe Gly Gln Cys Val Val Ala Gly Ala Lys Thr Thr Ala Asp Ala
 740 745 750
 Val Asp Ala Ala Gly Ala Pro Ala Asp Gly Thr Val Thr Leu Gly Arg
 755 760 765
 Trp Asn Ala Gly Val Arg Gly Gln Gly Arg Glu Ala Leu Phe Ser Arg
 770 775 780
 Thr Gln Gly Gly Met Val Ser Tyr Thr Phe Gly Glu Arg Glu Phe Val
 785 790 795 800
 Leu Arg Arg Pro Ser Ile Thr Thr Phe Arg Pro Leu Thr Asp Asn Asp
 805 810 815
 Arg Gly Ala Gly His Ala Phe Glu Arg Ala Ala Trp Ala Val Ala Gly
 820 825 830
 Lys Tyr Ala Arg Cys Val Asp Cys Ala Ile Ala Asn Arg Gly Glu Asn

835	840	845
Ala Val Glu Ala Thr Tyr Thr Tyr Glu Leu Ala Ile Pro Gln Arg Thr		
850	855	860
Lys Val Thr Val Arg Tyr Val Ala Asp Thr Ala Gly Leu Val Ser Leu		
865	870	875
Asp Val Glu Tyr Pro Gly Glu Lys Asn Gly Asp Leu Pro Thr Ile Pro		
885	890	895
Ala Phe Gly Ile Glu Trp Ala Leu Pro Val Glu Tyr Ala Asn Leu Arg		
900	905	910
Phe Tyr Gly Ala Gly Pro Glu Glu Thr Tyr Ala Asp Arg Arg His Ala		
915	920	925
Lys Leu Gly Val Trp Ser Thr Thr Ala Gly Asp Asp Cys Ala Pro Tyr		
930	935	940
Leu Leu Pro Gln Glu Thr Gly Asn His Glu Asp Val Arg Trp Ala Glu		
945	950	955
Ile Thr Asp Asp Ser Gly His Gly Val Arg Val Lys Arg Gly Ala Gly		
965	970	975
Ala Lys Pro Phe Ala Met Ser Leu Leu Pro Tyr Ser Ser Thr Met Leu		
980	985	990
Glu Glu Ala Leu His Gln Asp Glu Leu Pro Lys Pro Arg His Met Phe		
995	1000	1005
Leu Arg Leu Leu Ala Ala Gln Met Gly Val Gly Gly Asp Asp Ser		
1010	1015	1020
Trp Met Ser Pro Val His Glu Gln Tyr Gln Leu Pro Ala Asp Gln		
1025	1030	1035
Pro Leu Ser Leu Asn Val Gln Leu Lys Leu Phe		
1040	1045	
<210> 2		
<211> 625		
<212> PRT		
<213> 清酒乳杆菌 (Lactobacillus sakei)		
<400> 2		
Met Gln Pro Asn Ile Gln Trp Leu Asp Thr Pro Ala Val Phe Arg Val		
1	5	10
Gly Gln Leu Pro Ala His Ser Asp His Arg Tyr Tyr Ala Thr Leu Ala		
20	25	30
Glu Met Ala Gln Gln Gln Ser Ser Phe Glu Gln Ser Leu Asn Gly Thr		
35	40	45
Trp Gln Phe His Tyr Ser Val Asn Ala Ala Ser Arg Pro Lys Ser Phe		

50	55	60
Tyr Glu Leu Ala Phe Asp Ala Gln Asp Phe Glu Pro Ile Thr Val Pro		
65	70	75
Gln His Ile Glu Leu Ala Gly Tyr Glu Gln Leu His Tyr Ile Asn Thr		
	85	90
Met Tyr Pro Trp Glu Gly His Tyr Tyr Arg Arg Pro Ala Phe Ser Thr		
	100	105
Ser Asp Asp Lys Gln His Leu Gly Met Phe Ser Glu Ala Asp Tyr Asn		
	115	120
Pro Val Gly Ser Tyr Leu His His Phe Asp Leu Thr Pro Ala Leu Arg		
	130	135
Asn Gln Arg Val Ile Ile Arg Phe Glu Gly Val Glu Gln Ala Met Tyr		
145	150	155
Val Trp Leu Asn Gly Gln Phe Ile Gly Tyr Ala Glu Asp Ser Phe Thr		
	165	170
Pro Ser Glu Phe Asp Leu Thr Pro Tyr Leu Lys Glu Thr Asp Asn Cys		
	180	185
Leu Ala Val Glu Val His Lys Arg Ser Ser Ala Ala Phe Ile Glu Asp		
	195	200
Gln Asp Phe Phe Arg Phe Phe Gly Ile Phe Arg Asp Val Lys Leu Leu		
210	215	220
Ala Lys Pro Arg Thr His Leu Glu Asp Leu Trp Val Ile Pro Glu Tyr		
225	230	235
Asp Val Val Gln Gln Thr Gly Gln Val Lys Leu Arg Leu Gln Phe Ser		
	245	250
Gly Asp Glu Asn Arg Val His Leu Arg Ile Arg Asp Gln His Gln Ile		
	260	265
Ile Leu Thr Ala Asp Leu Thr Ser Ala Ala Gln Val Asn Gly Leu Tyr		
	275	280
Lys Met Pro Glu Leu Val Gln Ala Trp Ser Asn Gln Thr Pro Asn Leu		
	290	295
Tyr Thr Leu Glu Leu Glu Val Val Asp Gln Ala Gly Glu Thr Ile Glu		
305	310	315
Ile Ser Gln Gln Pro Phe Gly Phe Arg Lys Ile Glu Ile Lys Asp Lys		
	325	330
Val Met Leu Leu Asn Gly Lys Arg Leu Val Ile Asn Gly Val Asn Arg		
	340	345
His Glu Trp His Pro Glu Thr Gly Arg Thr Ile Thr Ala Glu Asp Glu		
	355	360
		365

Ala Trp Asp Ile Ala Cys Met Gln Arg Asn His Ile Asn Ala Val Arg
 370 375 380
 Thr Ser His Tyr Pro Asp Arg Leu Ser Phe Tyr Asn Gly Cys Asp Gln
 385 390 395 400
 Ala Gly Ile Tyr Met Met Ala Glu Thr Asn Leu Glu Ser His Gly Ser
 405 410 415
 Trp Gln Lys Met Gly Ala Val Glu Pro Ser Trp Asn Val Pro Gly Ser
 420 425 430
 Tyr Asp Glu Trp Glu Ala Ala Thr Leu Asp Arg Ala Arg Thr Asn Phe
 435 440 445
 Glu Thr Phe Lys Asn His Val Ser Ile Leu Phe Trp Ser Leu Gly Asn
 450 455 460
 Glu Ser Tyr Ala Gly Ser Val Leu Glu Lys Met Asn Ala Tyr Tyr Lys
 465 470 475 480
 Gln Gln Asp Pro Thr Arg Leu Val His Tyr Glu Gly Val Phe Arg Ala
 485 490 495
 Pro Glu Tyr Lys Ala Thr Ile Ser Asp Val Glu Ser Arg Met Tyr Ala
 500 505 510
 Thr Pro Ala Glu Ile Lys Ala Tyr Leu Asp Asn Ala Pro Gln Lys Pro
 515 520 525
 Phe Ile Leu Cys Glu Tyr Met His Asp Met Gly Asn Ser Leu Gly Gly
 530 535 540
 Met Gln Ser Tyr Ile Asp Leu Leu Ser Gln Tyr Asp Met Tyr Gln Gly
 545 550 555 560
 Gly Phe Ile Trp Asp Phe Ile Asp Gln Ala Leu Leu Val Thr Asp Pro
 565 570 575
 Val Thr Gly Gln Arg Glu Leu Arg Tyr Gly Gly Asp Phe Asp Asp Arg
 580 585 590
 Pro Ser Asp Tyr Glu Phe Ser Gly Asp Gly Leu Val Phe Ala Thr Arg
 595 600 605
 Asp Glu Lys Pro Ala Met Gln Glu Val Arg Tyr Tyr Tyr Gly Glu His
 610 615 620

Lys

625

<210> 3

<211> 330

<212> PRT

<213> 清酒乳杆菌

<400> 3

305	310	315	320
Asp His His Leu Glu Phe Glu Ile Ser Phe			
	325	330	
<210> 4			
<211> 1049			
<212> PRT			
<213> 青春双歧杆菌			
<400> 4			
Met Ala Asp Thr Ala Glu Leu Ala Ile Val His Ala Thr Thr Ala Ser			
1	5	10	15
Ala Ser Trp Leu Thr Asp Pro Thr Val Phe Ala Ala Asn Arg Lys Pro			
	20	25	30
Ala His Ser Ser His Arg Tyr Val Ile Gly Glu Thr Ser Glu Pro Lys			
	35	40	45
Gln Ser Leu Asp Gly Glu Trp Lys Val Arg Ile Glu Gln Ala Arg Asn			
	50	55	60
Val Asp Val Glu Ser Ala Pro Phe Ala Ala Val Asp Phe Glu Asp Gly			
65	70	75	80
Asp Phe Gly Ala Ile Glu Val Pro Gly His Leu Gln Met Ala Gly Tyr			
	85	90	95
Leu Lys Asn Lys Tyr Val Asn Ile Gln Tyr Pro Trp Asp Gly His Glu			
	100	105	110
Asp Pro Gln Ala Pro Asn Ile Pro Glu Asn Asn His Val Ala Ile Tyr			
	115	120	125
Arg Arg Arg Phe Ala Leu Asp Ala Gln Leu Ala Arg Thr Leu Glu Asn			
	130	135	140
Asp Gly Thr Val Ser Leu Thr Phe His Gly Ala Ala Thr Ala Ile Tyr			
145	150	155	160
Val Trp Leu Asp Gly Thr Phe Val Gly Tyr Gly Glu Asp Gly Phe Thr			
	165	170	175
Pro Ser Glu Phe Asp Val Thr Glu Ala Leu Arg Asn Gly Asn Gly Asn			
	180	185	190
Ala Ala Asp Ser Pro Glu Ala Glu His Thr Leu Thr Val Ala Cys Tyr			
	195	200	205
Glu Tyr Ser Ser Ala Ser Trp Leu Glu Asp Gln Asp Phe Trp Arg Leu			
	210	215	220
His Gly Leu Phe Arg Thr Val Glu Leu Ala Ala Gln Pro His Thr His			
225	230	235	240
Val Glu Thr Val Gln Leu Glu Ala Asp Tyr Thr Ala Ala Asp Thr Ala			

	245		250		255
Gly Thr Ala Asp Thr Ala Glu Leu Asn Ala Ala Leu Thr Leu Arg Asn					
	260		265		270
Pro Ala Asp Ala Met Thr Ile Glu Ser Thr Leu Arg Asp Gly Asp Gly					
	275		280		285
Asn Val Val Trp Glu Ser Thr Gln Ala Cys Asn Gly Glu Ile Ala Leu					
	290		295		300
Asn Ser Gly Lys Met Thr Asn Ile Ala Pro Trp Ser Ala Glu Ser Pro					
305		310		315	320
Thr Leu Tyr Thr Leu Thr Val Arg Val Val Gly His Asp Gly Ala Ile					
	325		330		335
Ile Glu Thr Val Thr Gln Lys Ile Gly Phe Arg Thr Phe Arg Ile Glu					
	340		345		350
Asn Gly Ile Met Thr Leu Asn Gly Lys Arg Ile Val Phe Lys Gly Ala					
	355		360		365
Asp Arg His Glu Phe Asp Ala Lys Arg Gly Arg Ala Ile Thr Arg Glu					
	370		375		380
Asp Met Leu Ser Asp Val Val Phe Cys Lys Arg His Asn Ile Asn Ala					
385		390		395	400
Ile Arg Thr Ser His Tyr Pro Asn Gln Glu Tyr Trp Tyr Asp Leu Cys					
	405		410		415
Asp Glu Tyr Gly Leu Tyr Leu Ile Asp Glu Thr Asn Met Glu Thr His					
	420		425		430
Gly Thr Trp Val Ala Asn Asn Val Glu Arg Pro Glu Asp Gly Ile Pro					
	435		440		445
Gly Ser Arg Pro Glu Trp Glu Gly Ala Cys Val Asp Arg Ile Asn Ser					
	450		455		460
Met Met Arg Arg Asp Tyr Asn His Pro Ser Val Leu Ile Trp Ser Leu					
465		470		475	480
Gly Asn Glu Ser Ser Ala Gly Glu Val Phe Arg Ala Met Tyr Arg His					
	485		490		495
Ala His Thr Ile Asp Pro Asn Arg Pro Val His Tyr Glu Gly Ser Val					
	500		505		510
His Met Arg Glu Phe Glu Asp Val Thr Asp Ile Glu Ser Arg Met Tyr					
	515		520		525
Ala His Ala Asp Glu Ile Glu Arg Tyr Leu Asn Asp Gly Ser Pro Ala					
	530		535		540
His Thr Asp Gly Pro Lys Lys Pro Tyr Ile Ser Cys Glu Tyr Met His					
545		550		555	560

Ala Met Gly Asn Ser Cys Gly Asn Met Asp Glu Tyr Thr Ala Leu Glu
 565 570 575
 Arg Tyr Pro Met Tyr Gln Gly Gly Phe Ile Trp Asp Phe Ile Asp Gln
 580 585 590
 Ala Ile Glu Thr Lys Leu Pro Asp Gly Thr Thr Arg Met Cys Tyr Gly
 595 600 605
 Gly Asp Phe Gly Asp Arg Pro Ser Asp Tyr Glu Phe Ser Gly Asp Gly
 610 615 620
 Leu Leu Phe Ala Asp Arg Thr Pro Ser Pro Lys Ala Gln Glu Val Lys
 625 630 635 640
 Gln Leu Tyr Ala Asn Val Lys Ile Ala Val Ser Val Asp Glu Ala Arg
 645 650 655
 Ile Thr Asn Asp Asn Leu Phe Val Ser Thr Gly Asp Tyr Arg Phe Val
 660 665 670
 Leu Arg Ile Leu Ala Asp Gly Lys Pro Val Trp Ser Thr Thr Arg Arg
 675 680 685
 Phe Asp Val Ala Ala Gly Glu Ser Ala Ser Phe Glu Val Asp Trp Pro
 690 695 700
 Val Asp Asp Tyr Arg Ser Asn Ala Glu Glu Leu Val Leu Glu Val Ser
 705 710 715 720
 Gln Gln Leu Gly Asn Ala Cys Asp Trp Ala Pro Ala Gly Tyr Glu Leu
 725 730 735
 Ala Phe Gly Gln Cys Val Val Ala Gly Ala Lys Thr Thr Ala Asp Ala
 740 745 750
 Val Asp Ala Ala Gly Ala Pro Ala Asp Gly Thr Val Thr Leu Gly Arg
 755 760 765
 Trp Asn Ala Gly Val Arg Gly Gln Gly Arg Glu Ala Leu Phe Ser Arg
 770 775 780
 Thr Gln Gly Gly Met Val Ser Tyr Thr Phe Gly Glu Arg Glu Phe Val
 785 790 795 800
 Leu Arg Arg Pro Ser Ile Thr Thr Phe Arg Pro Leu Thr Asp Asn Asp
 805 810 815
 Arg Gly Ala Gly His Ala Phe Glu Arg Ala Ala Trp Ala Val Ala Gly
 820 825 830
 Lys Tyr Ala Arg Cys Val Asp Cys Ala Ile Ala Asn Arg Gly Glu Asn
 835 840 845
 Ala Val Glu Ala Thr Tyr Thr Tyr Glu Leu Ala Ile Pro Gln Arg Thr
 850 855 860
 Lys Val Thr Val Arg Tyr Val Ala Asp Thr Ala Gly Leu Val Ser Leu

865		870		875		880
Asp Val Glu Tyr Pro Gly Glu Lys Asn Gly Asp Leu Pro Thr Ile Pro						
		885		890		895
Ala Phe Gly Ile Glu Trp Ala Leu Pro Val Glu Tyr Ala Asn Leu Arg						
		900		905		910
Phe Tyr Gly Ala Gly Pro Glu Glu Thr Tyr Ala Asp Arg Arg His Ala						
		915		920		925
Lys Leu Gly Val Trp Ser Thr Thr Ala Gly Asp Asp Cys Ala Pro Tyr						
		930		935		940
Leu Leu Pro Gln Glu Thr Gly Asn His Glu Asp Val Arg Trp Ala Glu						
945		950		955		960
Ile Thr Asp Asp Ser Gly His Gly Val Arg Val Lys Arg Gly Ala Gly						
		965		970		975
Ala Lys Pro Phe Ala Met Ser Leu Leu Pro Tyr Ser Ser Thr Met Leu						
		980		985		990
Glu Glu Ala Leu His Gln Asp Glu Leu Pro Lys Pro Arg His Met Phe						
		995		1000		1005
Leu Arg Leu Leu Ala Ala Gln Met Gly Val Gly Gly Asp Asp Ser						
1010		1015		1020		
Trp Met Ser Pro Val His Glu Gln Tyr Gln Leu Pro Ala Asp Gln						
1025		1030		1035		
Pro Leu Ser Leu Asn Val Gln Leu Lys Leu Phe						
1040		1045				
<210> 5						
<211> 626						
<212> PRT						
<213> 噬淀粉乳杆菌 (Lactobacillus amylovorus)						
<400> 5						
Met Lys Ala Asn Ile Lys Trp Leu Asp Asp Pro Glu Val Phe Arg Ile						
1		5		10		15
Asn Gln Leu Pro Ala His Ser Asp His Pro Phe Tyr Lys Asp Tyr Arg						
		20		25		30
Glu Trp Gln Asn His Ser Ser Ser Phe Lys Gln Ser Leu Asn Gly Ala						
		35		40		45
Trp Gln Phe His Phe Ser Lys Asp Pro Gln Ser Arg Pro Ile Asp Phe						
		50		55		60
Tyr Lys Arg Ser Phe Asp Ser Ser Ser Phe Asp Thr Ile Pro Val Pro						
65		70		75		80
Ser Glu Ile Glu Leu Asn Gly Tyr Ala Gln Asn Gln Tyr Thr Asn Ile						

				85					90					95	
Leu	Tyr	Pro	Trp	Glu	Ser	Lys	Ile	Tyr	Arg	Lys	Pro	Ala	Tyr	Thr	Leu
				100					105					110	
Gly	Arg	Gly	Ile	Lys	Asp	Gly	Asp	Phe	Ser	Gln	Gly	Lys	Asp	Asn	Thr
				115					120					125	
Val	Gly	Ser	Tyr	Leu	Lys	His	Phe	Asp	Leu	Asn	Pro	Ala	Leu	Ala	Gly
				130					135					140	
His	Asp	Ile	His	Ile	Gln	Phe	Glu	Gly	Val	Glu	Arg	Ala	Met	Tyr	Val
145					150					155					160
Tyr	Leu	Asn	Gly	His	Phe	Ile	Gly	Tyr	Ala	Glu	Asp	Ser	Phe	Thr	Pro
				165					170					175	
Ser	Glu	Phe	Asp	Leu	Thr	Pro	Tyr	Ile	Gln	Ala	Lys	Asp	Asn	Ile	Leu
				180					185					190	
Ala	Val	Glu	Val	Phe	Lys	His	Ser	Thr	Ala	Ser	Trp	Leu	Glu	Asp	Gln
				195					200					205	
Asp	Met	Phe	Arg	Phe	Ser	Gly	Ile	Phe	Arg	Ser	Val	Glu	Leu	Leu	Ala
				210					215					220	
Leu	Pro	Arg	Thr	His	Leu	Met	Asp	Leu	Asp	Ile	Lys	Pro	Thr	Val	Val
225					230					235					240
Asn	Asp	Tyr	His	Asp	Gly	Val	Phe	Asn	Ala	Lys	Leu	His	Phe	Met	Gly
				245					250					255	
Lys	Thr	Ser	Gly	Asn	Val	His	Val	Leu	Ile	Glu	Asp	Ile	Asp	Gly	Lys
				260					265					270	
Thr	Leu	Leu	Asn	Lys	Lys	Leu	Pro	Leu	Lys	Ser	Thr	Val	Glu	Ile	Glu
				275					280					285	
Asn	Glu	Thr	Phe	Ala	Asn	Val	His	Leu	Trp	Asp	Asn	His	Asp	Pro	Tyr
				290					295					300	
Leu	Tyr	Gln	Leu	Ile	Ile	Glu	Val	His	Asp	Gln	Asp	Gly	Lys	Leu	Val
305					310					315					320
Glu	Leu	Ile	Pro	Tyr	Gln	Phe	Gly	Phe	Arg	Lys	Ile	Glu	Ile	Thr	Lys
				325					330					335	
Asp	His	Val	Val	Leu	Leu	Asn	Gly	Lys	Arg	Leu	Ile	Ile	Asn	Gly	Val
				340					345					350	
Asn	Arg	His	Glu	Trp	Asp	Ala	Lys	Arg	Gly	Arg	Ser	Ile	Thr	Leu	Ala
				355					360					365	
Asp	Met	Lys	Gln	Asp	Ile	Ala	Thr	Phe	Lys	His	Asn	Asn	Ile	Asn	Ala
				370					375					380	
Val	Arg	Thr	Cys	His	Tyr	Pro	Asn	Gln	Ile	Pro	Trp	Tyr	Tyr	Leu	Cys
385					390					395					400

Asp Gln Asn Gly Ile Tyr Met Met Ala Glu Asn Asn Leu Glu Ser His
 405 410 415
 Gly Thr Trp Gln Lys Leu Gly Gln Val Glu Ala Thr Ser Asn Val Pro
 420 425 430
 Gly Ser Ile Pro Glu Trp Arg Glu Val Val Val Asp Arg Ala Arg Ser
 435 440 445
 Asn Tyr Glu Thr Phe Lys Asn His Thr Ala Ile Leu Phe Trp Ser Leu
 450 455 460
 Gly Asn Glu Ser Tyr Ala Gly Ser Asn Ile Ala Ala Met Asn Lys Leu
 465 470 475 480
 Tyr Lys Asp His Asp Ser Ser Arg Leu Thr His Tyr Glu Gly Val Phe
 485 490 495
 His Ala Pro Glu Phe Lys Lys Glu Ile Ser Asp Leu Glu Ser Cys Met
 500 505 510
 Tyr Leu Pro Pro Lys Glu Ala Glu Glu Tyr Leu Gln Asn Pro Lys Lys
 515 520 525
 Pro Leu Val Glu Cys Glu Tyr Met His Asp Met Gly Thr Pro Asp Gly
 530 535 540
 Gly Met Gly Ser Tyr Ile Lys Leu Ile Asp Lys Tyr Pro Gln Tyr Met
 545 550 555 560
 Gly Gly Phe Ile Trp Asp Phe Ile Asp Gln Ala Leu Leu Val His Asp
 565 570 575
 Pro Val Ser Gly Gln Asp Val Leu Arg Tyr Gly Gly Asp Phe Asp Asp
 580 585 590
 Arg His Ser Asp Tyr Glu Phe Ser Gly Asp Gly Leu Met Phe Ala Asp
 595 600 605
 Arg Thr Pro Lys Pro Ala Met Gln Glu Val Arg Tyr Tyr Tyr Gly Leu
 610 615 620
 His Lys
 625
 <210> 6
 <211> 316
 <212> PRT
 <213> 噬淀粉乳杆菌
 <400> 6
 Met Ala Tyr Thr Asn Asn Leu His Val Val Tyr Gly Glu Ala Ser Leu
 1 5 10 15
 Gly Val Asn Gly Gln Asp Phe Ala Tyr Leu Phe Ser Tyr Glu Arg Gly
 20 25 30

Gly Leu Glu Ser Leu Lys Ile Lys Asp Lys Glu Trp Leu Tyr Arg Thr
 35 40 45
 Pro Thr Pro Thr Phe Trp Arg Ala Thr Thr Asp Asn Asp Arg Gly Ser
 50 55 60
 Gly Phe Asn Gln Lys Ala Ala Gln Trp Leu Gly Ala Asp Met Phe Thr
 65 70 75 80
 Lys Cys Val Gly Ile His Val Gln Val Asp Asp His Arg Phe Asp Glu
 85 90 95
 Leu Pro Val Ala Pro Ile Asn Asn Gln Phe Ser Asn Gln Glu Phe Ala
 100 105 110
 His Glu Val Lys Val Ala Phe Asp Tyr Glu Thr Leu Thr Thr Pro Ala
 115 120 125
 Thr Lys Val Lys Ile Ile Tyr Asn Ile Asn Asp Phe Gly His Met Thr
 130 135 140
 Ile Thr Met His Tyr Phe Gly Lys Lys Gly Leu Pro Pro Leu Pro Val
 145 150 155 160
 Ile Gly Met Arg Phe Ile Met Pro Thr Lys Ala Lys Ser Phe Asp Tyr
 165 170 175
 Thr Gly Leu Ser Gly Glu Thr Tyr Pro Asp Arg Met Ala Gly Ala Glu
 180 185 190
 Arg Gly Thr Phe His Ile Asp Gly Leu Pro Val Thr Lys Tyr Leu Val
 195 200 205
 Pro Gln Glu Asn Gly Met His Met Gln Thr Asn Glu Leu Val Ile Thr
 210 215 220
 Arg Asn Ser Thr Gln Asn Asn Ala Asp Lys Asp Gly Asp Phe Ser Leu
 225 230 235 240
 Lys Ile Thr Gln Thr Lys Gln Pro Phe Asn Phe Ser Leu Leu Pro Tyr
 245 250 255
 Thr Ala Glu Glu Leu Glu Asn Ala Thr His Ile Glu Glu Leu Pro Leu
 260 265 270
 Ala Arg Arg Ser Val Leu Val Ile Ala Gly Ala Val Arg Gly Val Gly
 275 280 285
 Gly Ile Asp Ser Trp Gly Ser Asp Val Glu Glu Gln Tyr His Ile Asp
 290 295 300
 Pro Glu Gln Asp His Glu Phe Ser Phe Thr Leu Asn
 305 310 315
 <210> 7
 <211> 1052
 <212> PRT

<213> 两歧双歧杆菌 (Bifidobacterium bifidum)

<400> 7

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

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290	295	300
Lys Ala Asp Gly Thr Leu His Ala Glu Ala Glu Ile Asp Asp Ala Ala		
305	310	315
Pro Trp Ser Ala Glu Arg Pro Asp Leu Tyr Glu Leu Ser Val Thr Leu		
	325	330
Leu Asp Ala Asp Gly Lys Val Leu Glu Thr Ala Arg Thr Arg Ile Gly		
	340	345
Phe Arg His Val Ala Ile Glu Asp Gly Ile Leu Lys Leu Asn Gly Lys		
	355	360
Arg Leu Val Phe Arg Gly Val Asn Arg His Glu Phe Asp Cys Arg Arg		
	370	380
Gly Arg Ala Ile Thr Glu Glu Asp Met Leu Trp Asp Ile Arg Phe Met		
385	390	395
Lys Arg His Asn Ile Asn Ala Val Arg Thr Ser His Tyr Pro Asn Gln		
	405	410
Ser Arg Trp Tyr Glu Leu Cys Asp Glu Tyr Gly Ile Tyr Leu Ile Asp		
	420	425
Glu Thr Asn Leu Glu Thr His Gly Ser Trp Asn Ser Pro Gly Asp Ile		
	435	440
Pro Val Gly Thr Ser Val Pro Gly Asp Asp Glu Ala Trp Leu Gly Ala		
	450	455
Cys Ile Asp Arg Leu Asp Ser Met Ile Leu Arg Asp Arg Asn His Pro		
465	470	475
Ser Val Leu Val Trp Ser Leu Gly Asn Glu Ser Tyr Ala Gly Glu Val		
	485	490
Leu Lys Ala Met Ser Ala His Ala His Gln Leu Asp Pro Gly Arg Pro		
	500	505
Val His Tyr Glu Gly Val Asn Trp Asn His Ala Tyr Asp Gly Ile Ser		
	515	520
Asp Phe Glu Ser Arg Met Tyr Ala Lys Pro Ala Glu Ile Gln Asp Trp		
	530	535
Leu Glu His Gly Asp Glu Arg Gly Glu Ala Ser Lys Pro Phe Val Ser		
545	550	555
Cys Glu Tyr Met His Ala Met Gly Asn Ser Cys Gly Gly Leu Ser Glu		
	565	570
Phe Ile Asp Leu Glu Arg Tyr Glu Arg Tyr Ser Gly Gly Phe Ile Trp		
	580	585
Asp Tyr Ile Asp Gln Gly Leu Val Gln Arg Leu Pro Asp Gly Ser Glu		
	595	600
		605

Arg	Leu	Ser	Val	Gly	Gly	Glu	Trp	Gly	Asp	Arg	Pro	Thr	Asp	Tyr	Glu
610						615					620				
Phe	Val	Gly	Asn	Gly	Ile	Val	Phe	Ala	Asp	Arg	Thr	Pro	Ser	Pro	Lys
625					630					635					640
Ala	Gln	Glu	Val	Lys	Gln	Leu	Tyr	Ser	Pro	Val	Lys	Leu	Ala	Pro	Asp
				645						650					655
Gly	His	Gly	Val	Thr	Ile	Glu	Asn	Arg	Asn	Leu	Phe	Ala	Gly	Thr	Asp
			660						665						670
Gly	Tyr	Val	Phe	Ala	Ala	Arg	Leu	Leu	Glu	Asp	Gly	His	Glu	Ile	Trp
		675						680							685
His	Ala	Asp	Tyr	Arg	Phe	Asp	Val	Ala	Ala	Gly	Asp	Thr	Gln	His	His
		690					695								700
Asp	Ile	Ala	Phe	Pro	Asp	Ile	Asp	Ala	Asp	Gly	Asp	Thr	Arg	Glu	Val
705						710					715				720
Thr	Tyr	Glu	Val	Asp	Leu	Leu	Leu	Ala	Glu	Ala	Thr	Ala	Trp	Ala	Pro
				725							730				735
Ala	Gly	Tyr	Glu	Leu	Ala	Phe	Gly	Gln	Leu	Thr	Gly	Thr	Leu	Asn	Pro
			740												750
Glu	Gln	Asp	Ile	Thr	Glu	Thr	Ser	His	Asp	Asp	Asp	Gly	Arg	Ala	Thr
		755													765
Arg	Thr	Leu	Ser	Arg	Trp	Asn	Ala	Gly	Ile	Arg	Arg	Asp	Asp	Glu	Glu
		770					775								780
Ile	Leu	Leu	Ser	Arg	Thr	Gln	Gly	Gly	Ile	Val	Ser	Trp	Lys	Arg	Asp
785						790									800
Asp	Arg	Glu	Met	Val	Ile	Arg	Arg	Pro	Glu	Leu	Val	Thr	Phe	Arg	Pro
				805											815
Leu	Thr	Asp	Asn	Asp	Arg	Gly	Asn	His	Ser	Gly	Phe	Asp	Arg	Ala	Ala
			820												830
Trp	Phe	Ala	Ala	Gly	Arg	Tyr	Ala	Ile	Val	Thr	Glu	Thr	Lys	Ile	His
		835						840							845
Glu	Ser	Asp	Asp	Gly	Leu	Val	Ala	Glu	Tyr	Gln	Tyr	Glu	Leu	Ala	Asp
		850													860
Pro	Asn	His	Thr	Pro	Val	Ser	Val	Thr	Tyr	His	Val	Asn	Ser	Asp	Met
865							870								880
Arg	Met	Gln	Leu	Thr	Val	Glu	Tyr	Pro	Gly	Asn	Ala	Thr	Asp	Met	Ala
				885											895
Ser	Leu	Pro	Ala	Phe	Gly	Ile	Glu	Trp	Glu	Leu	Pro	Gly	Glu	Tyr	Asp
			900												910
Arg	Leu	Arg	Tyr	Tyr	Gly	Pro	Gly	Pro	Glu	Glu	Thr	Tyr	Arg	Asp	Arg

915	920	925
Lys Gln Gly Gly Lys Leu Gly Ile Trp Asp Ala Thr Ala Lys Ala Ser		
930	935	940
Met Ala Pro Tyr Leu Met Val Gln Glu Thr Gly Ser His Glu Asp Val		
945	950	955
Arg Trp Leu Glu Ala Thr Asp Ile Gln Gly His Gly Leu Arg Val Thr		
965	970	975
Gln Arg Gly Asp Arg His Phe Thr Ala Ser Leu Leu Pro Trp Asn Thr		
980	985	990
Tyr Thr Ile Glu Ala Ala Arg Arg His Glu Asp Leu Pro Lys Pro Arg		
995	1000	1005
His Asn Tyr Leu Arg Leu Leu Ala Ala Gln Met Gly Val Gly Gly		
1010	1015	1020
Asp Asp Ser Trp Gly Ala Pro Val His Thr Ala Tyr Gln Leu Pro		
1025	1030	1035
Ala Gly Arg Pro Leu Thr Leu Asp Val Asn Leu Glu Leu Ile		
1040	1045	1050
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<211> 1052		
<212> PRT		
<213> 两歧双歧杆菌		
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Met Asn Thr Thr Asp Asp Gln Arg Lys Asn Gly Asp Pro Ile Val Ser		
1	5	10
Pro Ser Ile Pro Thr Thr Ala Trp Leu Ala Asp Pro Arg Val Tyr Ala		
20	25	30
Val His Arg Leu Asp Ala His Ser Asp His Ala Cys Trp Ser Arg Ser		
35	40	45
Pro Val Asp Gly Glu Ser Thr Asp Leu Arg Gln Ser Leu Asp Gly Glu		
50	55	60
Trp Arg Val Arg Val Glu Thr Ala Pro Thr Gly Arg Phe Pro Asp Gly		
65	70	75
Thr Ser Asp Gly Pro Asp Trp Ile Ser Asp Val Ser Pro Leu Phe Ala		
85	90	95
Ala Pro Gly Phe Asp Asp Ser Ser Phe Ser Arg Val Gln Val Pro Ser		
100	105	110
His Leu Glu Thr Ala Gly Leu Leu Ala Pro Gln Tyr Val Asn Val Gln		
115	120	125
Tyr Pro Trp Asp Gly His Glu Asp Pro Lys Ala Pro Ala Ile Pro Glu		

130	135	140
His Gly His Val Ala Val Tyr Arg Arg Glu Phe Asp Ala Asp Gly Glu		
145	150	155
Val Ala Gln Ala Val Arg Glu Gly Arg Pro Val Thr Leu Thr Phe Gln		
	165	170
Gly Ala Ala Thr Ala Ile Tyr Val Trp Leu Asn Gly Ser Phe Ile Gly		
	180	185
Tyr Ala Glu Asp Ser Phe Thr Pro Ser Glu Phe Asp Val Thr Asp Ala		
	195	200
Ile Lys Val Asp Gly Asn Val Leu Ala Val Ala Cys Tyr Glu Tyr Ser		
210	215	220
Ser Ala Ser Trp Leu Glu Asp Gln Asp Phe Trp Arg Leu His Gly Leu		
225	230	235
Phe Arg Ser Val Glu Leu Asn Ala Arg Pro Ala Ala His Val Ala Asp		
	245	250
Leu His Ala Asp Ala Asp Trp Asp Leu Ala Thr Ser Arg Gly Ser Leu		
	260	265
Ser Leu Asp Val Leu Ile Asp Gly Ala Ala Asn Ala Ala Thr Ala Asp		
275	280	285
Phe Ala Leu Trp Asp Lys Asn Gly Thr Ile Val Trp His Ile Val Thr		
290	295	300
Lys Ala Asp Gly Thr Leu His Ala Glu Ala Glu Ile Asp Asp Ala Ala		
305	310	315
Pro Trp Ser Ala Glu Arg Pro Asp Leu Tyr Glu Leu Ser Val Thr Leu		
	325	330
Leu Asp Ala Asp Gly Lys Val Leu Glu Thr Ala Arg Thr Arg Ile Gly		
	340	345
Phe Arg His Val Ala Ile Glu Asp Gly Ile Leu Lys Leu Asn Gly Lys		
	355	360
Arg Leu Val Phe Arg Gly Val Asn Arg His Glu Phe Asp Cys Arg Arg		
370	375	380
Gly Arg Ala Ile Thr Glu Glu Asp Met Leu Trp Asp Ile Arg Phe Met		
385	390	395
Lys Arg His Asn Ile Asn Ala Val Arg Thr Ser His Tyr Pro Asn Gln		
	405	410
Ser Arg Trp Tyr Glu Leu Cys Asp Glu Tyr Gly Ile Tyr Leu Ile Asp		
	420	425
Glu Thr Asn Leu Glu Thr His Gly Ser Trp Asn Ser Pro Gly Asp Ile		
435	440	445

Pro Val Gly Thr Ser Val Pro Gly Asp Asp Glu Ala Trp Leu Gly Ala
 450 455 460
 Cys Ile Asp Arg Leu Asp Ser Met Ile Leu Arg Asp Arg Asn His Pro
 465 470 475 480
 Ser Val Leu Val Trp Ser Leu Gly Asn Glu Ser Tyr Ala Gly Glu Val
 485 490 495
 Leu Lys Ala Met Ser Ala His Ala His Arg Leu Asp Pro Gly Arg Pro
 500 505 510
 Val His Tyr Glu Gly Val Asn Trp Asn His Ala Tyr Asp Gly Ile Ser
 515 520 525
 Asp Phe Glu Ser Arg Met Tyr Ala Lys Pro Ala Glu Ile Gln Asp Trp
 530 535 540
 Leu Glu His Gly Asp Glu Arg Gly Glu Ala Ser Lys Pro Phe Val Ser
 545 550 555 560
 Cys Glu Tyr Met His Ala Met Gly Asn Ser Cys Gly Gly Leu Ser Glu
 565 570 575
 Phe Ile Asp Leu Glu Arg Tyr Glu Arg Tyr Ser Gly Gly Phe Ile Trp
 580 585 590
 Asp Tyr Ile Asp Gln Gly Leu Val Gln Arg Leu Pro Asp Gly Ser Glu
 595 600 605
 Arg Leu Ser Val Gly Gly Glu Trp Gly Asp Arg Pro Thr Asp Tyr Glu
 610 615 620
 Phe Val Gly Asn Gly Ile Val Phe Ala Asp Arg Thr Pro Ser Pro Lys
 625 630 635 640
 Ala Gln Glu Val Lys Gln Leu Tyr Ser Pro Val Lys Leu Ala Pro Asp
 645 650 655
 Gly His Gly Val Thr Ile Glu Asn Arg Asn Leu Phe Ala Gly Thr Asp
 660 665 670
 Gly Tyr Val Phe Ala Ala Arg Leu Leu Glu Asp Gly His Glu Ile Trp
 675 680 685
 His Ala Asp Tyr Arg Phe Asp Val Ala Ala Gly Asp Thr Gln His His
 690 695 700
 Asp Ile Ala Phe Pro Asp Ile Asp Ala Asp Gly Asp Thr Arg Glu Val
 705 710 715 720
 Thr Tyr Glu Val Asp Leu Leu Leu Ala Glu Ala Thr Ala Trp Ala Pro
 725 730 735
 Ala Gly Tyr Glu Leu Ala Phe Gly Gln Leu Thr Gly Thr Leu Asn Pro
 740 745 750
 Glu Gln Asp Ile Thr Glu Thr Ser His Asp Asp Asp Gly Arg Ala Thr

755	760	765
Arg Thr Leu Ser Arg Trp Asn Ala Gly Ile Arg Arg Asp Asp Lys Glu		
770	775	780
Ile Leu Leu Ser Arg Thr Gln Gly Gly Ile Val Ser Trp Lys Arg Asp		
785	790	795
Asp Arg Glu Met Val Ile Arg Arg Pro Glu Leu Val Thr Phe Arg Pro		
805	810	815
Leu Thr Asp Asn Asp Arg Gly Asn His Ser Gly Phe Asp Arg Ala Ala		
820	825	830
Trp Phe Ala Ala Gly Arg Tyr Ala Ile Val Thr Glu Thr Lys Ile His		
835	840	845
Glu Ser Asp Asp Gly Leu Val Ala Glu Tyr Gln Tyr Glu Leu Ala Asp		
850	855	860
Pro Asn His Thr Pro Val Ser Val Thr Tyr His Val Asn Ser Asp Met		
865	870	875
Arg Met Gln Leu Thr Val Glu Tyr Pro Gly Asn Ala Thr Asp Met Ala		
885	890	895
Ser Leu Pro Ala Phe Gly Ile Glu Trp Glu Leu Pro Gly Glu Tyr Asp		
900	905	910
Arg Leu Arg Tyr Tyr Gly Pro Gly Pro Glu Glu Thr Tyr Arg Asp Arg		
915	920	925
Lys Gln Gly Gly Lys Leu Gly Ile Trp Asp Ala Thr Ala Lys Ala Ser		
930	935	940
Met Ala Pro Tyr Leu Met Val Gln Glu Thr Gly Ser His Glu Asp Val		
945	950	955
Arg Trp Leu Glu Ala Thr Asp Ile Gln Gly His Gly Leu Arg Val Thr		
965	970	975
Gln Arg Gly Asp Arg His Phe Thr Ala Ser Leu Leu Pro Trp Asn Thr		
980	985	990
Tyr Met Ile Glu Ala Ala Arg Arg His Glu Asp Leu Pro Glu Pro Arg		
995	1000	1005
His Asn Tyr Leu Arg Leu Leu Ala Ala Gln Met Gly Val Gly Gly		
1010	1015	1020
Asp Asp Ser Trp Gly Ala Pro Val His Thr Ala Tyr Gln Leu Pro		
1025	1030	1035
Ala Gly Arg Pro Leu Thr Leu Asp Val Asn Leu Glu Leu Ile		
1040	1045	1050
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<212> PRT

<213> 短双歧杆菌 (Bifidobacterium breve)

<400> 9

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

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Ala Ala Thr Val Leu Asp Ala Ala Gly Asn Thr Val Trp His Ser Lys
 290 295 300
 Leu Thr Ala Gly Ala Asp Ala Glu Thr Leu Thr Val Lys Ala Asn Val
 305 310 315 320
 Gly Lys Val Asn Pro Trp Ser Ala Glu Glu Pro Thr Leu Tyr Thr Leu
 325 330 335
 Gln Val Val Ala Thr Asp Ala Ala Gly Gln Val Ile Glu Ala Ala Leu
 340 345 350
 Gln Arg Ile Gly Phe Arg His Phe Ala Ile Glu Asp Gly Leu Met Lys
 355 360 365
 Leu Asn Gly Lys Arg Ile Val Phe Lys Gly Val Asp Arg His Glu Phe
 370 375 380
 Asp Ala Arg Thr Gly Arg Thr Ile Ala Glu Ala Asp Met Ile Glu Asp
 385 390 395 400
 Ile His Ser Phe Lys Arg Leu Asn Ile Asn Ala Val Arg Thr Ser His
 405 410 415
 Tyr Pro Asn Glu Thr Arg Trp Tyr Glu Leu Cys Asp Glu Tyr Gly Ile
 420 425 430
 Tyr Val Leu Asp Glu Thr Asn Leu Glu Thr His Gly Ser Trp Thr Asp
 435 440 445
 Pro Gly Asp Val Phe Gln Pro Ala Arg Ala Ile Pro Gly Ser Lys Asp
 450 455 460
 Glu Trp Arg Ala Ala Cys Val Asp Arg Thr Ala Ser Met Val Arg Arg
 465 470 475 480
 Asp Tyr Asn His Pro Ser Val Val Ile Trp Ser Leu Gly Asn Glu Ala
 485 490 495
 Phe Gly Gly Asp Val Phe Tyr Ser Met Arg Asp Phe Val His Glu Asn
 500 505 510
 Asp Pro Phe Arg Pro Val His Tyr Glu Gly Thr Phe Asn Asp Pro Glu
 515 520 525
 Phe Ser Ala Ala Thr Asp Ile Met Ser Arg Met Tyr Ala Lys Pro Asp
 530 535 540
 Glu Ile Val Lys Leu Tyr Leu Gly Glu Asp Gly Lys Lys Pro Tyr Ile
 545 550 555 560
 Ser Cys Glu Tyr Ser His Ser Met Gly Asn Ser Thr Gly Gly Leu His
 565 570 575
 Leu Tyr Thr Glu Leu Glu Arg Tyr Pro Leu Tyr Gln Gly Gly Phe Ile
 580 585 590
 Trp Asp Tyr Val Asp Gln Ala Leu Trp Gln Asp Cys Gly Asn Gly Thr

595	600	605
Glu Arg Leu Ala Tyr Gly Gly Asp Phe Glu Asp Arg Pro Asn Asp Tyr		
610	615	620
Glu Phe Ser Gly Asp Gly Val Met Phe Ala Asp Arg Thr Pro Ser Pro		
625	630	635
Lys Ala Gln Glu Val Lys Gln Leu Tyr Ala Asn Val Lys Leu Val Pro		
645	650	655
Asp Glu Ser Gly Val Thr Ile Thr Asn Asp Asn Leu Phe Ile Ser Thr		
660	665	670
Ala Ser Ser Leu Phe Thr Ala Arg Val Leu Val Asp Gly Ala Glu Arg		
675	680	685
Trp His Ala Asn Tyr Arg Phe Asp Val Pro Ala Gly Glu Thr Val Arg		
690	695	700
Glu Pro Ile Ala Phe Pro Lys Val Thr Asp Leu Val Ala Leu Ser Gly		
705	710	715
Ser Ala Glu Val Thr Tyr Glu Val Asp Gln Arg Leu Ala Glu Ala Thr		
725	730	735
Asp Trp Ala Pro Ala Gly Tyr Glu Leu Thr Phe Gly Gln Tyr Val Ala		
740	745	750
Ala Val Ser Phe Asp Asp Gly Ala Ala Asp Ala Val Val Ala Gly Asp		
755	760	765
Ala Glu Val Ala Ala Asp Gly Phe Asn Ala Gly Ile His Thr Asp Phe		
770	775	780
Gly Glu Val Leu Leu Ser Lys Thr Gln Gly Gly Met Val Ser Phe Lys		
785	790	795
Arg Asp Gly Arg Glu Met Val Ile Arg Arg Pro Asn Leu Thr Thr Phe		
805	810	815
Arg Ala Leu Thr Asp Asn Asp Arg Gly Asn Gly Ser Gly Phe Glu Arg		
820	825	830
Ala Gln Trp Met Ala Ala Gly Arg Tyr Ala Arg Val Thr Gly Thr Ser		
835	840	845
Val Glu Glu Thr Ala Asp Gly Lys Gly Leu Lys Ala Thr Tyr Ser Tyr		
850	855	860
Glu Leu Ala Asp Ala Lys His Thr Pro Val Thr Val His Tyr Glu Val		
865	870	875
Asp Ala Ala Leu Arg Val His Leu Thr Val Glu Tyr Pro Gly Glu Ala		
885	890	895
Asp Ala Ala Thr Leu Pro Ala Phe Gly Leu Glu Trp Ile Leu Pro Lys		
900	905	910

Gln Tyr Asp Arg Leu Arg Phe Tyr Gly Leu Gly Pro Glu Glu Thr Tyr
 915 920 925
 Ala Asp Arg Leu His Gly Ala Lys Leu Gly Val Phe Ser Arg Thr Ala
 930 935 940
 Ala Glu Asp Cys Ala Pro Tyr Leu Leu Pro Gln Glu Thr Gly Asn His
 945 950 955 960
 Glu Gln Val Arg Trp Ala Glu Ile Thr Asp Glu Tyr Gly His Gly Met
 965 970 975
 Arg Val Thr Ala Ala Gly Gly Thr Arg Phe Ala Thr Ser Leu Leu Pro
 980 985 990
 Tyr Ser Ser Leu Met Phe Glu Asp Ala Leu His Gln Asn Glu Leu Pro
 995 1000 1005
 Lys Pro Arg His Thr Phe Leu Arg Leu Leu Ala Ala Gln Met Gly
 1010 1015 1020
 Val Gly Gly Asp Asp Thr Trp Gly Ala Pro Val His Asp Glu Phe
 1025 1030 1035
 Gln Val Pro Ala Asp Gln Pro Leu Lys Leu Asp Val Thr Leu Glu
 1040 1045 1050
 Leu Ile
 1055
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 <211> 689
 <212> PRT
 <213> 链状双歧杆菌 (*Bifidobacterium catenulatum*)
 <400> 10
 Met Thr Gln Arg Arg Ser Tyr Arg Trp Pro Gln Pro Leu Ala Gly Gln
 1 5 10 15
 Gln Ala Arg Ile Trp Tyr Gly Gly Asp Tyr Asn Pro Asp Gln Trp Pro
 20 25 30
 Glu Glu Val Trp Asp Asp Asp Val Arg Leu Met Lys Lys Ala Gly Val
 35 40 45
 Asn Leu Val Ser Val Gly Ile Phe Ser Trp Ala Lys Ile Glu Thr Ser
 50 55 60
 Glu Gly Val Tyr Asp Phe Asp Trp Leu Asp Arg Ile Ile Asp Lys Leu
 65 70 75 80
 Gly Glu Ala Gly Ile Ala Val Asp Leu Ala Ser Ala Thr Ala Ser Pro
 85 90 95
 Pro Met Trp Leu Thr Gln Ala His Pro Glu Val Leu Trp Lys Asp Tyr
 100 105 110

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

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

Gln

<210> 11

<211> 1023

<212> PRT

<213> 链状双歧杆菌

<400> 11

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

305	310	315	320
Ile Val Glu Ala Ala Val Gln Lys Val Gly Phe Arg Arg Phe Arg Ile			
	325	330	335
Glu Asn Gly Ile Met Thr Leu Asn Gly Lys Arg Ile Val Phe Lys Gly			
	340	345	350
Ala Asp Arg His Glu Phe Asp Ala Lys Arg Gly Arg Ser Ile Thr Glu			
	355	360	365
Gln Asp Met Ile Asp Asp Val Ile Phe Cys Lys Arg His Asn Ile Asn			
	370	375	380
Ala Ile Arg Thr Ser His Tyr Pro Asn Gln Glu Arg Trp Tyr Asp Leu			
385	390	395	400
Cys Asp Glu Tyr Gly Ile Tyr Leu Ile Asp Glu Thr Asn Leu Glu Thr			
	405	410	415
His Gly Ser Trp Cys Leu Pro Gly Asp Val Val Thr Ala Glu Thr Ala			
	420	425	430
Val Pro Gly Ser Lys Ala His Trp Glu Gly Ala Cys Val Asp Arg Val			
	435	440	445
Asn Ser Met Val Arg Arg Asp Tyr Asn His Pro Ser Val Val Ile Trp			
	450	455	460
Ser Leu Gly Asn Glu Ser Tyr Thr Gly Asp Val Phe Arg Ala Met Tyr			
465	470	475	480
Lys His Val His Asp Ile Asp Pro Asn Arg Pro Val His Tyr Glu Gly			
	485	490	495
Val Thr Lys Asn Arg Asp Tyr Asp Asp Val Thr Asp Ile Glu Thr Arg			
	500	505	510
Met Tyr Glu His Ala Asp Val Val Glu Glu Tyr Leu Lys Asn Asp Pro			
	515	520	525
Gln Lys Pro Tyr Ile Ser Cys Glu Tyr Met His Ala Met Gly Asn Ser			
	530	535	540
Val Gly Asn Leu Asp Glu Tyr Thr Ala Leu Glu Arg Tyr Pro His Tyr			
545	550	555	560
Gln Gly Gly Phe Ile Trp Asp Phe Ile Asp Gln Ala Ile Tyr Ala Thr			
	565	570	575
Gln Pro Asp Gly Ser Thr Arg Leu Cys Tyr Gly Gly Asp Phe Gly Asp			
	580	585	590
Arg Pro Ser Asp Tyr Glu Phe Ser Gly Asn Gly Leu Val Phe Ala Asp			
	595	600	605
Arg Thr Pro Thr Pro Lys Ala Gln Glu Val Lys Gln Leu Tyr Ser Asn			
	610	615	620

Val His Ile Asp Val Thr Asp Arg Ser Val Ser Ile Lys Asn Asp Asn																			
625																			
Leu Phe Ile Ser Thr Gly Gly Tyr Gln Phe Val Leu Arg Ile Leu Ala																			
Asp Gly Glu Pro Val Trp Gln Ser Glu Arg Arg Phe Asp Val Pro Ala																			
Asp Ser Ala Cys Thr Phe Asp Val Glu Trp Pro Val Asp Leu Tyr Arg																			
Ala Asn Ala Asp Glu Leu Val Leu Glu Val Ser Gln Arg Leu Ala Glu																			
Ala Thr Asp Trp Ala Pro Ala Gly Tyr Glu Leu Ala Phe Gly Gln Thr																			
Ile Val Ala Gly Thr Lys Ala Ala Glu Asp Ala Ala Leu Pro Ala Asp																			
Gly Ile Val Thr Val Gly Arg Trp Asn Ala Gly Val Gln Gly Ser Gly																			
Arg Glu Ile Leu Leu Ser Arg Thr Gln Gly Gly Leu Val Ser Tyr Thr																			
Phe Asp Gly His Glu Phe Val Leu Arg Arg Pro Ala Ile Thr Thr Phe																			
Arg Ala Leu Thr Asp Asn Asp Arg Gly Ala Gly His Gly Phe Glu Arg																			
Ala Gln Trp Met Val Ala Gly Arg Tyr Ala Arg Cys Val Asp Asn Val																			
Ile Glu Gln Val Asp Glu Asp Thr Leu Lys Ala Val Tyr Thr Tyr Glu																			
Leu Ala Thr Pro Gln Cys Thr Lys Val Thr Val Gly Tyr Thr Ala Asp																			
Thr Thr Gly Arg Leu Asn Leu His Val Glu Tyr Pro Gly Glu Ser Gly																			
Glu Leu Pro Thr Ile Pro Ala Phe Gly Ile Glu Trp Thr Leu Pro Val																			
Gln Tyr Ser Asn Leu Arg Phe Phe Gly Ala Gly Pro Glu Glu Thr Tyr																			
Gln Asp Arg Lys His Ala Lys Leu Gly Val Trp Ser Thr Asp Ala Phe																			
Lys Asp His Ala Pro Tyr Leu Met Pro Gln Glu Thr Gly Asn His Glu																			
Glu Val Arg Trp Ala Glu Ile Thr Asp Glu Asn Gly His Gly Leu Arg																			

930	935	940
Val Ser Arg Ala Asn Gly	Ala Ala Pro Phe Ala	Val Ser Leu Gln Pro
945	950	955
Tyr Ser Ser Phe Met Ile	Glu Glu Ala Gln His	Gln Asp Glu Leu Pro
	965	970
Ala Pro Lys His Met Phe	Leu Arg Val Leu Ala	Ala Gln Met Gly Val
	980	985
Gly Gly Asp Asp Ser Trp	Met Ser Pro Val His	Ser Gln Tyr His Ile
	995	1000
Thr Ala Asp Gln Pro Ile	Ser Leu Asp Val Asn	Leu Glu Leu Ile
	1010	1015
		1020
<210> 12		
<211> 1008		
<212> PRT		
<213> 德氏乳杆菌保加利亚亚种 (<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>)		
<400> 12		
Met Ser Asn Lys Leu Val	Lys Glu Lys Arg Val	Asp Gln Ala Asp Leu
1	5	10
Ala Trp Leu Thr Asp Pro	Glu Val Tyr Glu Val	Asn Thr Ile Pro Pro
	20	25
His Ser Asp His Glu Ser	Phe Gln Ser Gln Glu	Glu Leu Glu Glu Gly
	35	40
Lys Ser Ser Leu Val Gln	Ser Leu Asp Gly Asp	Trp Leu Ile Asp Tyr
	50	55
Ala Glu Asn Gly Gln Gly	Pro Val Asn Phe Tyr	Ala Glu Asp Phe Asp
65	70	75
Asp Ser Asn Phe Lys Ser	Val Lys Val Pro Gly	Asn Leu Glu Leu Gln
	85	90
Gly Phe Gly Gln Pro Gln	Tyr Val Asn Val Gln	Tyr Pro Trp Asp Gly
	100	105
Ser Glu Glu Ile Phe Pro	Pro Gln Ile Pro Ser	Lys Asn Pro Leu Ala
	115	120
Ser Tyr Val Arg Tyr Phe	Asp Leu Asp Glu Ala	Phe Trp Asp Lys Glu
	130	135
Val Ser Leu Lys Phe Asp	Gly Ala Ala Thr Ala	Ile Tyr Val Trp Leu
145	150	155
Asn Gly His Phe Val Gly	Tyr Gly Glu Asp Ser	Phe Thr Pro Ser Glu
	165	170
Phe Met Val Thr Lys Phe	Leu Lys Lys Glu Asn	Asn Arg Leu Ala Val

180	185	190
Ala Leu Tyr Lys Tyr Ser Ser	Ala Ser Trp Leu Glu Asp Gln Asp Phe	
195	200	205
Trp Arg Met Ser Gly Leu Phe Arg Ser Val Thr Leu Gln Ala Lys Pro		
210	215	220
Arg Leu His Leu Glu Asp Leu Lys Leu Thr Ala Ser Leu Thr Asp Asn		
225	230	235
240	245	250
Tyr Gln Lys Gly Lys Leu Glu Val Glu Ala Asn Ile Ala Tyr Arg Leu		
255	260	265
Pro Asn Ala Ser Phe Lys Leu Glu Val Arg Asp Ser Glu Gly Asp Leu		
270	275	280
Val Ala Glu Lys Leu Gly Pro Ile Arg Ser Glu Gln Leu Glu Phe Thr		
285	290	295
Leu Ala Asp Leu Pro Val Ala Ala Trp Ser Ala Glu Lys Pro Asn Leu		
300	305	310
Tyr Gln Val Arg Leu Tyr Leu Tyr Gln Ala Gly Ser Leu Leu Glu Val		
315	320	325
Ser Arg Gln Glu Val Gly Phe Arg Asn Phe Glu Leu Lys Asp Gly Ile		
330	335	340
Met Tyr Leu Asn Gly Gln Arg Ile Val Phe Lys Gly Ala Asn Arg His		
345	350	355
Glu Phe Asp Ser Lys Leu Gly Arg Ala Ile Thr Glu Glu Asp Met Ile		
360	365	370
Trp Asp Ile Lys Thr Met Lys Arg Ser Asn Ile Asn Ala Val Arg Cys		
375	380	385
Ser His Tyr Pro Asn Gln Ser Leu Phe Tyr Arg Leu Cys Asp Lys Tyr		
390	395	400
Gly Leu Tyr Val Ile Asp Glu Ala Asn Leu Glu Ser His Gly Thr Trp		
405	410	415
Glu Lys Val Gly Gly His Glu Asp Pro Ser Phe Asn Val Pro Gly Asp		
420	425	430
Asp Gln His Trp Leu Gly Ala Ser Leu Ser Arg Val Lys Asn Met Met		
435	440	445
Ala Arg Asp Lys Asn His Ala Ser Ile Leu Ile Trp Ser Leu Gly Asn		
450	455	460
Glu Ser Tyr Ala Gly Thr Val Phe Ala Gln Met Ala Asp Tyr Val Arg		
465	470	475
480	485	490
Lys Ala Asp Pro Thr Arg Val Gln His Tyr Glu Gly Val Thr His Asn		
495		

Arg Lys Phe Asp Asp Ala Thr Gln Ile Glu Ser Arg Met Tyr Ala Pro
 500 505 510
 Ala Lys Val Ile Glu Glu Tyr Leu Thr Asn Lys Pro Ala Lys Pro Phe
 515 520 525
 Ile Ser Val Glu Tyr Ala His Ala Met Gly Asn Ser Val Gly Asp Leu
 530 535 540
 Ala Ala Tyr Thr Ala Leu Glu Lys Tyr Pro His Tyr Gln Gly Gly Phe
 545 550 555 560
 Ile Trp Asp Trp Ile Asp Gln Gly Leu Glu Lys Asp Gly His Leu Leu
 565 570 575
 Tyr Gly Gly Asp Phe Asp Asp Arg Pro Thr Asp Tyr Glu Phe Cys Gly
 580 585 590
 Asn Gly Leu Val Phe Ala Asp Arg Thr Glu Ser Pro Lys Leu Ala Asn
 595 600 605
 Val Lys Ala Leu Tyr Ala Asn Leu Lys Leu Glu Val Lys Asp Gly Gln
 610 615 620
 Leu Phe Leu Lys Asn Asp Asn Leu Phe Thr Asn Ser Ser Ser Tyr Tyr
 625 630 635 640
 Phe Leu Thr Ser Leu Leu Val Asp Gly Lys Leu Thr Tyr Gln Ser Arg
 645 650 655
 Pro Leu Thr Phe Gly Leu Glu Pro Gly Glu Ser Gly Thr Phe Ala Leu
 660 665 670
 Pro Trp Pro Glu Val Ala Asp Glu Lys Gly Glu Val Val Tyr Arg Val
 675 680 685
 Thr Ala His Leu Lys Glu Asp Leu Pro Trp Ala Asp Glu Gly Phe Thr
 690 695 700
 Val Ala Glu Ala Glu Glu Val Ala Gln Lys Leu Pro Glu Phe Lys Pro
 705 710 715 720
 Glu Gly Arg Pro Asp Leu Val Asp Ser Asp Tyr Asn Leu Gly Leu Lys
 725 730 735
 Gly Asn Asn Phe Gln Ile Leu Phe Ser Lys Val Lys Gly Trp Pro Val
 740 745 750
 Ser Leu Lys Tyr Ala Gly Arg Glu Tyr Leu Lys Arg Leu Pro Glu Phe
 755 760 765
 Thr Phe Trp Arg Ala Leu Thr Asp Asn Asp Arg Gly Ala Gly Tyr Gly
 770 775 780
 Tyr Asp Leu Ala Arg Trp Glu Asn Ala Gly Lys Tyr Ala Arg Leu Lys
 785 790 795 800
 Asp Ile Ser Cys Glu Val Lys Glu Asp Ser Val Leu Val Lys Thr Ala

	805	810	815
Phe Thr Leu Pro Val Ala Leu Lys Gly Asp Leu Thr Val Thr Tyr Glu			
	820	825	830
Val Asp Gly Arg Gly Lys Ile Ala Val Thr Ala Asp Phe Pro Gly Ala			
	835	840	845
Glu Glu Ala Gly Leu Leu Pro Ala Phe Gly Leu Asn Leu Ala Leu Pro			
	850	855	860
Lys Glu Leu Thr Asp Tyr Arg Tyr Tyr Gly Leu Gly Pro Asn Glu Ser			
865	870	875	880
Tyr Pro Asp Arg Leu Glu Gly Asn Tyr Leu Gly Ile Tyr Gln Gly Ala			
	885	890	895
Val Lys Lys Asn Phe Ser Pro Tyr Leu Arg Pro Gln Glu Thr Gly Asn			
	900	905	910
Arg Ser Lys Val Arg Trp Tyr Gln Leu Phe Asp Glu Lys Gly Gly Leu			
	915	920	925
Glu Phe Thr Ala Asn Gly Ala Asp Leu Asn Leu Ser Ala Leu Pro Tyr			
	930	935	940
Ser Ala Ala Gln Ile Glu Ala Ala Asp His Ala Phe Asp Leu Thr Asn			
945	950	955	960
Asn Tyr Thr Trp Val Arg Ala Leu Ser Ala Gln Met Gly Val Gly Gly			
	965	970	975
Asp Asp Ser Trp Gly Gln Lys Val His Pro Glu Phe Cys Leu Asp Ala			
	980	985	990
Gln Lys Ala Arg Gln Leu Arg Leu Val Ile Gln Pro Leu Leu Leu Lys			
	995	1000	1005
<210> 13			
<211> 1007			
<212> PRT			
<213> 德氏乳杆菌乳酸亚种 (<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>)			
<400> 13			
Met Ser Asn Lys Leu Val Lys Glu Lys Arg Val Asp Gln Ala Asp Leu			
1	5	10	15
Ala Trp Leu Thr Asp Pro Glu Val Tyr Glu Val Asn Thr Ile Pro Pro			
	20	25	30
His Ser Asp His Glu Ser Phe Gln Ser Gln Glu Glu Leu Glu Glu Gly			
	35	40	45
Lys Ser Ser Leu Val Gln Ser Leu Asp Gly Asn Trp Leu Ile Asp Tyr			
	50	55	60
Ala Glu Asn Gly Gln Gly Pro Ile Asn Phe Tyr Ala Glu Asp Phe Asp			

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

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

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

<210> 14
 <211> 1008
 <212> PRT
 <213> 德氏乳杆菌保加利亚亚种
 <400> 14
 Met Ser Asn Lys Leu Val Lys Glu Lys Arg Val Asp Gln Ala Asp Leu
 1 5 10 15
 Ala Trp Leu Thr Asp Pro Glu Val Tyr Glu Val Asn Thr Ile Pro Pro
 20 25 30
 His Phe Asp His Glu Ser Phe Gln Ser Gln Glu Glu Leu Glu Glu Gly
 35 40 45
 Lys Ser Ser Leu Val Gln Ser Leu Asp Gly Asp Trp Leu Ile Asp Tyr
 50 55 60
 Ala Glu Asn Gly Gln Gly Pro Val Asn Phe Tyr Ala Glu Asp Phe Asp
 65 70 75 80
 Asp Ser Asn Phe Lys Ser Val Lys Val Pro Gly Asn Leu Glu Leu Gln
 85 90 95
 Gly Phe Gly Gln Pro Gln Tyr Val Asn Val Gln Tyr Pro Trp Asp Gly
 100 105 110
 Ser Glu Glu Ile Phe Pro Pro Gln Ile Pro Ser Lys Asn Pro Leu Ala
 115 120 125
 Ser Tyr Val Arg Tyr Phe Asp Leu Asp Glu Ala Phe Trp Asp Lys Glu
 130 135 140
 Val Ser Leu Lys Phe Asp Gly Ala Ala Thr Ala Ile Tyr Val Trp Leu
 145 150 155 160
 Asn Gly His Phe Val Gly Tyr Gly Glu Asp Ser Phe Thr Pro Ser Glu
 165 170 175
 Phe Met Val Thr Lys Phe Leu Lys Lys Glu Asn Asn Arg Leu Ala Val
 180 185 190
 Ala Leu Tyr Lys Tyr Ser Ser Ala Ser Trp Leu Glu Asp Gln Asp Phe
 195 200 205
 Trp Arg Met Ser Gly Leu Phe Arg Ser Val Thr Leu Gln Ala Lys Pro
 210 215 220
 Arg Leu His Leu Glu Asp Leu Lys Leu Thr Ala Ser Leu Thr Asp Asn
 225 230 235 240
 Tyr Gln Lys Gly Lys Leu Glu Val Glu Ala Asn Ile Ala Tyr Arg Leu
 245 250 255
 Pro Asn Ala Ser Phe Lys Leu Glu Val Arg Asp Ser Glu Gly Asp Leu
 260 265 270

Val Ala Glu Lys Leu Gly Pro Ile Arg Ser Glu Gln Leu Glu Phe Thr
 275 280 285
 Leu Ala Asp Leu Pro Val Ala Ala Trp Ser Ala Glu Lys Pro Asn Leu
 290 295 300
 Tyr Gln Val Arg Leu Tyr Leu Tyr Gln Ala Gly Ser Leu Leu Glu Val
 305 310 315 320
 Ser Arg Gln Glu Val Gly Phe Arg Asn Phe Glu Leu Lys Asp Gly Ile
 325 330 335
 Met Tyr Leu Asn Gly Gln Arg Ile Val Phe Lys Gly Ala Asn Arg His
 340 345 350
 Glu Phe Asp Ser Lys Leu Gly Arg Ala Ile Thr Glu Glu Asp Met Ile
 355 360 365
 Trp Asp Ile Lys Thr Met Lys Arg Ser Asn Ile Asn Ala Val Arg Cys
 370 375 380
 Ser His Tyr Pro Asn Gln Ser Leu Phe Tyr Arg Leu Cys Asp Lys Tyr
 385 390 395 400
 Gly Leu Tyr Val Ile Asp Glu Ala Asn Leu Glu Ser His Gly Thr Trp
 405 410 415
 Glu Lys Val Gly Gly His Glu Asp Pro Ser Phe Asn Val Pro Gly Asp
 420 425 430
 Asp Gln His Trp Leu Gly Ala Ser Leu Ser Arg Val Lys Asn Met Met
 435 440 445
 Ala Arg Asp Lys Asn His Ala Ser Ile Leu Ile Trp Ser Leu Gly Asn
 450 455 460
 Glu Ser Tyr Ala Gly Thr Val Phe Ala Gln Met Ala Asp Tyr Val Arg
 465 470 475 480
 Lys Ala Asp Pro Thr Arg Val Gln His Tyr Glu Gly Val Thr His Asn
 485 490 495
 Arg Lys Phe Asp Asp Ala Thr Gln Ile Glu Ser Arg Met Tyr Ala Pro
 500 505 510
 Ala Lys Val Ile Glu Glu Tyr Leu Thr Asn Lys Pro Ala Lys Pro Phe
 515 520 525
 Ile Ser Val Glu Tyr Ala His Ala Met Gly Asn Ser Val Gly Asp Leu
 530 535 540
 Ala Ala Tyr Thr Ala Leu Glu Lys Tyr Pro His Tyr Gln Gly Gly Phe
 545 550 555 560
 Ile Trp Asp Trp Ile Asp Gln Gly Leu Glu Lys Asp Gly His Leu Leu
 565 570 575
 Tyr Gly Gly Asp Phe Asp Asp Arg Pro Thr Asp Tyr Glu Phe Cys Gly

580	585	590
Asn Gly Leu Val Phe Ala Asp Arg Thr Glu Ser Pro Lys Leu Ala Asn		
595	600	605
Val Lys Ala Leu Tyr Ala Asn Leu Lys Leu Glu Val Lys Asp Gly Gln		
610	615	620
Leu Phe Leu Lys Asn Asp Asn Leu Phe Thr Asn Ser Ser Ser Tyr Tyr		
625	630	635
Phe Leu Thr Ser Leu Leu Val Asp Gly Lys Leu Thr Tyr Gln Ser Arg		
645	650	655
Pro Leu Thr Phe Gly Leu Glu Pro Gly Glu Ser Gly Thr Phe Ala Leu		
660	665	670
Pro Trp Pro Glu Val Ala Asp Glu Lys Gly Glu Val Val Tyr Arg Val		
675	680	685
Thr Ala His Leu Lys Glu Asp Leu Pro Trp Ala Asp Glu Gly Phe Thr		
690	695	700
Val Ala Glu Ala Glu Glu Val Ala Gln Lys Leu Pro Glu Phe Lys Pro		
705	710	715
Glu Gly Arg Pro Asp Leu Val Asp Ser Asp Tyr Asn Leu Gly Leu Lys		
725	730	735
Gly Asn Asn Phe Gln Ile Leu Phe Ser Lys Val Lys Gly Trp Pro Val		
740	745	750
Ser Leu Lys Tyr Ala Gly Arg Glu Tyr Leu Lys Arg Leu Pro Glu Phe		
755	760	765
Thr Phe Trp Arg Ala Leu Thr Asp Asn Asp Arg Gly Ala Gly Tyr Gly		
770	775	780
Tyr Asp Leu Ala Arg Trp Glu Asn Ala Gly Lys Tyr Ala Arg Leu Lys		
785	790	795
Asp Ile Ser Cys Glu Val Lys Glu Asp Ser Val Leu Val Lys Thr Ala		
805	810	815
Phe Thr Leu Pro Val Ala Leu Lys Gly Asp Leu Thr Val Thr Tyr Glu		
820	825	830
Val Asp Gly Arg Gly Lys Ile Ala Val Thr Ala Asp Phe Pro Gly Ala		
835	840	845
Glu Glu Ala Gly Leu Leu Pro Ala Phe Gly Leu Asn Leu Ala Leu Pro		
850	855	860
Lys Glu Leu Thr Asp Tyr Arg Tyr Tyr Gly Leu Gly Pro Asn Glu Ser		
865	870	875
Tyr Pro Asp Arg Leu Glu Gly Asn Tyr Leu Gly Ile Tyr Gln Gly Ala		
885	890	895

Val Lys Lys Asn Phe Ser Pro Tyr Leu Arg Pro Gln Glu Thr Gly Asn
 900 905 910
 Arg Ser Lys Val Arg Trp Tyr Gln Leu Phe Asp Glu Lys Gly Gly Leu
 915 920 925
 Glu Phe Thr Ala Asn Gly Ala Asp Leu Asn Leu Ser Ala Leu Pro Tyr
 930 935 940
 Ser Ala Ala Gln Ile Glu Ala Ala Asp His Ala Phe Glu Leu Thr Asn
 945 950 955 960
 Asn Tyr Thr Trp Val Arg Ala Leu Ser Ala Gln Met Gly Val Gly Gly
 965 970 975
 Asp Asp Ser Trp Gly Gln Lys Val His Pro Glu Phe Cys Leu Asp Ala
 980 985 990
 Gln Lys Ala Arg Gln Leu Arg Leu Val Ile Gln Pro Leu Leu Leu Lys
 995 1000 1005
 <210> 15
 <211> 1008
 <212> PRT
 <213> 德氏乳杆菌保加利亚亚种
 <400> 15
 Met Ser Asn Lys Leu Val Lys Glu Lys Arg Val Asp Gln Ala Asp Leu
 1 5 10 15
 Ala Trp Leu Thr Asp Pro Glu Val Tyr Glu Val Asn Thr Ile Pro Pro
 20 25 30
 His Ser Asp His Glu Ser Phe Gln Ser Gln Glu Glu Leu Glu Glu Gly
 35 40 45
 Lys Ser Ser Leu Val Gln Ser Leu Asp Gly Asp Trp Leu Ile Asp Tyr
 50 55 60
 Ala Glu Asn Gly Gln Gly Pro Val Asn Phe Tyr Ala Glu Asp Phe Asp
 65 70 75 80
 Asp Ser Asn Phe Lys Ser Val Lys Val Pro Gly Asn Leu Glu Leu Gln
 85 90 95
 Gly Phe Gly Gln Pro Gln Tyr Val Asn Val Gln Tyr Pro Trp Asp Gly
 100 105 110
 Ser Glu Glu Ile Phe Pro Pro Gln Ile Pro Ser Lys Asn Pro Leu Ala
 115 120 125
 Ser Tyr Val Arg Tyr Phe Asp Leu Asp Glu Ala Phe Trp Asp Lys Glu
 130 135 140
 Val Ser Leu Lys Phe Asp Gly Ala Ala Thr Ala Ile Tyr Val Trp Leu
 145 150 155 160

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

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

Lys Ser Ser Leu Val Gln Ser Leu Asp Gly Asp Trp Leu Ile Asp Tyr
 50 55 60
 Ala Glu Asn Gly Gln Gly Pro Val Asn Phe Tyr Ala Glu Asp Phe Asp
 65 70 75 80
 Asp Ser Asn Phe Lys Ser Val Lys Val Pro Gly Asn Leu Glu Leu Gln
 85 90 95
 Gly Phe Gly Gln Pro Gln Tyr Val Asn Ile Gln Tyr Pro Trp Asp Gly
 100 105 110
 Ser Glu Glu Ile Phe Pro Pro Gln Val Pro Ser Lys Asn Pro Leu Ala
 115 120 125
 Ser Tyr Val Arg Tyr Phe Asp Leu Asp Glu Ala Phe Trp Asp Lys Glu
 130 135 140
 Val Ser Leu Lys Phe Ala Gly Ala Ala Thr Ala Ile Tyr Val Trp Leu
 145 150 155 160
 Asn Gly His Phe Val Gly Tyr Gly Glu Asp Ser Phe Thr Pro Ser Glu
 165 170 175
 Phe Met Val Thr Lys Phe Leu Lys Lys Glu Asn Asn Arg Leu Ala Val
 180 185 190
 Ala Leu Tyr Lys Tyr Ser Ser Ala Ser Trp Leu Glu Asp Gln Asp Phe
 195 200 205
 Trp Arg Leu Ser Gly Leu Phe Arg Ser Val Thr Leu Gln Ala Lys Pro
 210 215 220
 Leu Leu His Leu Glu Asp Leu Lys Leu Thr Ala Ser Leu Thr Asp Asn
 225 230 235 240
 Tyr Gln Lys Gly Lys Leu Glu Val Glu Ala Asn Ile Ala Tyr Arg Leu
 245 250 255
 Pro Asn Ala Ser Phe Lys Leu Glu Val Arg Asp Ser Glu Gly Asp Leu
 260 265 270
 Val Ala Glu Lys Leu Gly Pro Ile Arg Ser Glu Gln Leu Glu Phe Thr
 275 280 285
 Leu Ala Asp Leu Pro Val Ala Ala Trp Ser Ala Glu Lys Pro Asn Leu
 290 295 300
 Tyr Gln Val Arg Leu Tyr Leu Tyr Gln Ala Gly Ser Leu Leu Glu Val
 305 310 315 320
 Ser Arg Gln Glu Val Gly Phe Arg Asn Phe Glu Leu Lys Asp Gly Ile
 325 330 335
 Met Tyr Leu Asn Gly Gln Arg Ile Val Phe Lys Gly Val Asn Arg His
 340 345 350
 Glu Phe Asp Ser Lys Leu Gly Arg Ala Ile Thr Glu Glu Asp Met Ile

355	360	365
Trp Asp Ile Lys Thr Met Lys Arg Ser Asn Ile Asn Ala Val Arg Cys		
370	375	380
Ser His Tyr Pro Asn Gln Ser Leu Phe Tyr Arg Leu Cys Asp Lys Tyr		
385	390	395
Gly Leu Tyr Val Ile Asp Glu Ala Asn Leu Glu Ser His Gly Thr Trp		
405	410	415
Glu Lys Val Gly His Glu Asp Pro Ser Phe Asn Val Pro Gly Asp Asp		
420	425	430
Gln His Trp Leu Gly Ala Ser Leu Ser Arg Val Lys Asn Met Met Ala		
435	440	445
Arg Asp Lys Asn His Ala Ser Ile Leu Ile Trp Ser Leu Gly Asn Glu		
450	455	460
Ser Tyr Ala Gly Thr Val Phe Ala Gln Met Ala Asp Tyr Val Arg Lys		
465	470	475
Ala Asp Pro Thr Arg Val Gln His Tyr Glu Gly Val Thr His Asn Arg		
485	490	495
Lys Phe Asp Asp Ala Thr Gln Ile Glu Ser Arg Met Tyr Ala Pro Ala		
500	505	510
Lys Glu Ile Glu Glu Tyr Leu Thr Lys Lys Pro Ala Lys Pro Phe Ile		
515	520	525
Ser Val Glu Tyr Ala His Ala Met Gly Asn Ser Val Gly Asp Leu Ala		
530	535	540
Ala Tyr Thr Ala Leu Glu Lys Tyr Pro His Tyr Gln Gly Gly Phe Ile		
545	550	555
Trp Asp Trp Ile Asp Gln Gly Leu Glu Lys Asp Gly His Leu Leu Tyr		
565	570	575
Gly Gly Gly Asp Phe Asp Asp Arg Pro Thr Asp Tyr Glu Phe Cys Gly		
580	585	590
Asn Gly Leu Val Phe Ala Asp Arg Thr Thr Ser Pro Lys Leu Ala Asn		
595	600	605
Val Lys Ala Leu Tyr Ser Asn Leu Lys Leu Glu Val Lys Asp Gly Gln		
610	615	620
Leu Phe Leu Lys Asn Asp Asn Leu Phe Thr Asn Ser Ser Ala Tyr Tyr		
625	630	635
Phe Leu Thr Ser Leu Leu Val Asp Gly Lys Leu Thr Tyr Gln Ser Gln		
645	650	655
Pro Leu Thr Phe Gly Leu Glu Pro Gly Glu Ser Gly Thr Phe Val Leu		
660	665	670

Pro Trp Pro Glu Val Glu Asp Glu Lys Gly Glu Ile Val Tyr Gln Val
 675 680 685
 Thr Ala His Leu Lys Glu Asp Leu Pro Trp Ala Asp Glu Gly Phe Thr
 690 695 700
 Val Ala Glu Ala Glu Glu Ala Val Thr Lys Leu Pro Glu Phe Tyr Pro
 705 710 715 720
 Ala Gly Arg Pro Glu Leu Val Asp Ser Asp Phe Asn Leu Gly Leu Lys
 725 730 735
 Gly Asn Gly Phe Arg Ile Leu Phe Ser Lys Ala Lys Gly Trp Pro Val
 740 745 750
 Ser Ile Lys Tyr Ala Gly Arg Glu Tyr Leu Lys Arg Leu Pro Glu Phe
 755 760 765
 Thr Phe Trp Arg Ala Leu Thr Asp Asn Asp Arg Gly Ala Gly Tyr Gly
 770 775 780
 Tyr Asp Leu Ala Lys Trp Glu Asn Ala Gly Lys Tyr Ala Arg Leu Gln
 785 790 795 800
 Asp Ile Ser Tyr Glu Ile Lys Glu Asn Ser Val Leu Val Lys Thr Ala
 805 810 815
 Phe Thr Leu Pro Val Ala Leu Lys Gly Asp Leu Thr Ile Thr Tyr Glu
 820 825 830
 Val Asp Ser Leu Gly Lys Ile Ala Val Thr Ala Asn Phe Pro Gly Ala
 835 840 845
 Val Glu Asn Gly Leu Leu Pro Ala Phe Gly Leu Asn Phe Ala Leu Pro
 850 855 860
 Lys Glu Leu Ser Asp Tyr Arg Tyr Tyr Gly Leu Gly Pro Asn Glu Ser
 865 870 875 880
 Tyr Ala Asp Arg Leu Glu Gly Ser Tyr Leu Gly Ile Tyr Gln Gly Ala
 885 890 895
 Val Glu Lys Asn Phe Thr Pro Tyr Leu Arg Pro Gln Glu Ala Gly Asn
 900 905 910
 Arg Ser Lys Val Arg Tyr Tyr Gln Leu Phe Asp Glu Glu Ser Gly Leu
 915 920 925
 Glu Phe Thr Ala Asn Gly Ala Asp Leu Asn Leu Ser Ala Leu Pro Tyr
 930 935 940
 Ser Ala Ala Gln Ile Glu Ala Ala Asp His Ala Phe Glu Leu Ser Asn
 945 950 955 960
 Asn Tyr Thr Trp Val Arg Ala Leu Ala Ala Gln Met Gly Val Gly Gly
 965 970 975
 Asp Asp Ser Trp Gly Gln Lys Val His Pro Glu Phe Cys Leu Asp Ala

980			985			990									
Gln	Glu	Ala	Arg	Gln	Leu	Lys	Leu	Val	Ile	Gln	Pro	Leu	Leu	Leu	Lys
		995			1000			1005							
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1			5						10					15	
Ala	Trp	Leu	Thr	Asp	Pro	Glu	Val	Tyr	Glu	Val	Asn	Thr	Ile	Pro	Pro
		20						25						30	
His	Ser	Asp	His	Glu	Ser	Phe	Gln	Ser	Gln	Glu	Glu	Leu	Glu	Glu	Gly
		35						40						45	
Lys	Ser	Ser	Leu	Val	Gln	Ser	Leu	Asp	Gly	Asp	Trp	Leu	Ile	Asp	Tyr
		50						55						60	
Ala	Glu	Asn	Gly	Gln	Gly	Pro	Val	Asn	Phe	Tyr	Ala	Glu	Asp	Phe	Asp
65					70						75				80
Asp	Ser	Asn	Phe	Lys	Ser	Val	Lys	Val	Pro	Gly	Asn	Leu	Glu	Leu	Gln
					85						90				95
Gly	Phe	Gly	Gln	Pro	Gln	Tyr	Val	Asn	Val	Gln	Tyr	Pro	Trp	Asp	Gly
					100						105				110
Ser	Glu	Glu	Ile	Phe	Pro	Pro	Gln	Ile	Pro	Ser	Lys	Asn	Pro	Leu	Ala
					115									125	
Ser	Tyr	Val	Arg	Tyr	Phe	Asp	Leu	Asp	Glu	Ala	Phe	Trp	Asp	Lys	Glu
		130						135						140	
Val	Ser	Leu	Lys	Phe	Asp	Gly	Ala	Ala	Thr	Ala	Ile	Tyr	Val	Trp	Leu
145					150						155				160
Asn	Gly	His	Phe	Val	Gly	Tyr	Gly	Glu	Asp	Ser	Phe	Thr	Pro	Ser	Glu
					165						170				175
Phe	Met	Val	Thr	Lys	Phe	Leu	Lys	Lys	Glu	Asn	Asn	Arg	Leu	Ala	Val
					180									190	
Ala	Leu	Tyr	Lys	Tyr	Ser	Ser	Ala	Ser	Trp	Leu	Glu	Asp	Gln	Asp	Phe
		195						200						205	
Trp	Arg	Met	Ser	Gly	Leu	Phe	Arg	Ser	Val	Thr	Leu	Gln	Ala	Lys	Pro
		210						215						220	
Arg	Leu	His	Leu	Glu	Asp	Leu	Lys	Leu	Thr	Ala	Ser	Leu	Thr	Asp	Asn
225					230						235				240
Tyr	Gln	Lys	Gly	Lys	Leu	Glu	Val	Glu	Ala	Asn	Ile	Ala	Tyr	Arg	Leu

	245	250	255
Pro Asn Ala Ser Phe Lys Leu Glu Val Arg Asp Ser Glu Gly Asp Leu			
	260	265	270
Val Ala Glu Lys Leu Gly Pro Ile Arg Ser Glu Gln Leu Glu Phe Thr			
	275	280	285
Leu Ala Asp Leu Pro Val Ala Ala Trp Ser Ala Glu Lys Pro Asn Leu			
	290	295	300
Tyr Gln Val Arg Leu Tyr Leu Tyr Gln Ala Gly Ser Leu Leu Glu Val			
305	310	315	320
Ser Arg Gln Glu Val Gly Phe Arg Asn Phe Glu Leu Lys Asp Gly Ile			
	325	330	335
Met Tyr Leu Asn Gly Gln Arg Ile Val Phe Lys Gly Val Asn Arg His			
	340	345	350
Glu Phe Asp Ser Lys Leu Gly Arg Ala Ile Thr Glu Glu Asp Met Ile			
	355	360	365
Trp Asp Ile Lys Thr Ile Lys Arg Ser Asn Ile Asn Ala Val Arg Cys			
	370	375	380
Ser His Tyr Pro Asn Gln Ser Leu Phe Tyr Arg Leu Cys Asp Lys Tyr			
385	390	395	400
Gly Leu Tyr Val Ile Asp Glu Ala Asn Leu Glu Ser His Gly Thr Trp			
	405	410	415
Glu Lys Val Gly Gly His Glu Asp Pro Ser Phe Asn Val Pro Gly Asp			
	420	425	430
Asp Gln His Trp Leu Gly Ala Ser Leu Ser Arg Val Lys Asn Met Met			
	435	440	445
Ala Arg Asp Lys Asn His Ala Ser Ile Leu Ile Trp Ser Leu Gly Asn			
	450	455	460
Glu Ser Tyr Ala Gly Thr Val Phe Ala Gln Met Ala Asp Tyr Val Arg			
465	470	475	480
Lys Ala Asp Pro Thr Arg Val Gln His Tyr Glu Gly Val Thr His Asn			
	485	490	495
Arg Lys Phe Asp Asp Ala Thr Gln Ile Glu Ser Arg Met Tyr Ala Pro			
	500	505	510
Ala Lys Val Ile Glu Glu Tyr Leu Thr Asn Lys Pro Ala Lys Pro Phe			
	515	520	525
Ile Ser Val Glu Tyr Ala His Ala Met Gly Asn Ser Val Gly Asp Leu			
	530	535	540
Ala Ala Tyr Thr Ala Leu Glu Lys Tyr Pro His Tyr Gln Gly Gly Phe			
545	550	555	560

Ile Trp Asp Trp Ile Asp Gln Gly Leu Glu Lys Asp Gly His Leu Leu
 565 570 575
 Tyr Gly Gly Asp Phe Asp Asp Arg Pro Thr Asp Tyr Glu Phe Cys Gly
 580 585 590
 Asn Gly Leu Val Phe Ala Asp Arg Thr Glu Ser Pro Lys Leu Ala Asn
 595 600 605
 Val Lys Ala Leu Tyr Ala Asn Leu Lys Leu Glu Val Lys Asp Gly Gln
 610 615 620
 Leu Phe Leu Lys Asn Asp Asn Leu Phe Thr Asn Ser Ser Ser Tyr Tyr
 625 630 635 640
 Phe Leu Thr Ser Leu Leu Val Asp Gly Lys Leu Thr Tyr Gln Ser Arg
 645 650 655
 Pro Leu Thr Phe Gly Leu Glu Pro Gly Glu Ser Gly Thr Phe Ala Leu
 660 665 670
 Pro Trp Pro Glu Val Ala Asp Glu Lys Gly Glu Val Val Tyr Arg Val
 675 680 685
 Thr Ala His Leu Lys Glu Asp Leu Pro Trp Ala Asp Glu Gly Phe Thr
 690 695 700
 Val Ala Glu Ala Glu Glu Val Ala Gln Lys Leu Pro Glu Phe Lys Pro
 705 710 715 720
 Glu Gly Arg Pro Asp Leu Val Asp Ser Asp Tyr Asn Leu Gly Leu Lys
 725 730 735
 Gly Asn Asn Phe Gln Ile Leu Phe Ser Lys Val Lys Gly Trp Pro Val
 740 745 750
 Ser Leu Lys Tyr Ala Gly Arg Glu Tyr Leu Lys Arg Leu Pro Glu Phe
 755 760 765
 Thr Phe Trp Arg Ala Leu Thr Asp Asn Asp Arg Gly Ala Gly Tyr Gly
 770 775 780
 Tyr Asp Leu Ala Arg Trp Glu Asn Ala Gly Lys Tyr Ala Arg Leu Lys
 785 790 795 800
 Asp Ile Ser Cys Glu Val Lys Glu Asp Ser Val Leu Val Lys Thr Ala
 805 810 815
 Phe Thr Leu Pro Val Ala Leu Lys Gly Asp Leu Thr Val Thr Tyr Glu
 820 825 830
 Val Asp Gly Arg Gly Lys Ile Ala Val Thr Ala Asp Phe Pro Gly Ala
 835 840 845
 Glu Glu Ala Gly Leu Leu Pro Ala Phe Gly Leu Asn Leu Ala Leu Pro
 850 855 860
 Lys Glu Leu Thr Asp Tyr Arg Tyr Tyr Gly Leu Gly Pro Asn Glu Ser

865	870	875	880
Tyr Pro Asp Arg Leu Glu Gly Asn Tyr Leu Gly Ile Tyr Gln Gly Ala			
	885	890	895
Val Lys Lys Asn Phe Ser Pro Tyr Leu Arg Pro Gln Glu Thr Gly Asn			
	900	905	910
Arg Ser Lys Val Arg Trp Tyr Gln Leu Phe Asp Glu Lys Gly Gly Leu			
	915	920	925
Glu Phe Thr Ala Asn Gly Ala Asp Leu Asn Leu Ser Ala Leu Pro Tyr			
	930	935	940
Ser Ala Ala Gln Ile Glu Ala Ala Asp His Ala Phe Asp Leu Thr Asn			
945	950	955	960
Asn Tyr Thr Trp Val Arg Ala Leu Ser Ala Gln Met Gly Val Gly Gly			
	965	970	975
Asp Asp Ser Trp Gly Gln Lys Val His Pro Glu Phe Cys Leu Asp Ala			
	980	985	990
Gln Lys Ala Arg Gln Leu Arg Leu Val Ile Gln Pro Leu Leu Leu Lys			
	995	1000	1005
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<212> PRT			
<213> 德氏乳杆菌保加利亚亚种			
<400> 18			
Met Ser Asn Lys Leu Val Lys Glu Lys Arg Val Asp Gln Ala Asp Leu			
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Ala Trp Leu Thr Asp Pro Glu Val Tyr Glu Val Asn Thr Ile Pro Pro			
	20	25	30
His Ser Asp His Glu Ser Phe Gln Ser Gln Glu Glu Leu Glu Glu Gly			
	35	40	45
Lys Ser Ser Leu Val Gln Ser Leu Asp Gly Asp Trp Leu Ile Asp Tyr			
	50	55	60
Ala Glu Asn Gly Gln Gly Pro Val Asn Phe Tyr Ala Glu Asp Phe Asp			
65	70	75	80
Asp Ser Asn Phe Lys Ser Val Lys Val Pro Gly Asn Leu Glu Leu Gln			
	85	90	95
Gly Phe Gly Gln Pro Gln Tyr Val Asn Val Gln Tyr Pro Trp Asp Gly			
	100	105	110
Ser Glu Glu Ile Phe Pro Pro Gln Ile Pro Ser Lys Asn Pro Leu Ala			
	115	120	125
Ser Tyr Val Arg Tyr Phe Asp Leu Asp Glu Ala Phe Trp Asp Lys Glu			

130	135	140
Val Ser Leu Lys Phe Asp Gly Ala Ala Thr Ala Ile Tyr Val Trp Leu		
145	150	155
Asn Gly His Phe Val Gly Tyr Gly Glu Asp Ser Phe Thr Pro Ser Glu		
	165	170
Phe Met Val Thr Lys Phe Leu Lys Lys Glu Asn Asn Arg Leu Ala Val		
	180	185
Ala Leu Tyr Lys Tyr Ser Ser Ala Ser Trp Leu Glu Asp Gln Asp Phe		
	195	200
Trp Arg Met Ser Gly Leu Phe Arg Ser Val Thr Leu Gln Ala Lys Pro		
210	215	220
Arg Leu His Leu Glu Asp Leu Lys Leu Thr Ala Ser Leu Thr Asp Asn		
225	230	235
Tyr Gln Lys Gly Lys Leu Glu Val Glu Ala Asn Ile Ala Tyr Arg Leu		
	245	250
Pro Asn Ala Ser Phe Lys Leu Glu Val Arg Asp Ser Glu Gly Asp Leu		
	260	265
Val Ala Glu Lys Leu Gly Pro Ile Gly Ser Glu Gln Leu Glu Phe Thr		
275	280	285
Leu Ala Asp Leu Pro Val Ala Ala Trp Ser Ala Glu Lys Pro Asn Leu		
290	295	300
Tyr Gln Val Arg Leu Tyr Leu Tyr Gln Ala Gly Ser Leu Leu Glu Val		
305	310	315
Ser Arg Gln Glu Val Gly Phe Arg Asn Phe Glu Leu Lys Asp Gly Ile		
	325	330
Met Tyr Leu Asn Gly Gln Arg Ile Val Phe Lys Gly Val Asn Arg His		
	340	345
Glu Phe Asp Ser Lys Leu Gly Arg Ala Ile Thr Glu Glu Asp Met Ile		
	355	360
Trp Asp Ile Lys Thr Ile Lys Arg Ser Asn Ile Asn Ala Val Arg Cys		
370	375	380
Ser His Tyr Pro Asn Gln Ser Leu Phe Tyr Arg Leu Cys Asp Lys Tyr		
385	390	395
Gly Leu Tyr Val Ile Asp Glu Ala Asn Leu Glu Ser His Gly Thr Trp		
	405	410
Glu Lys Val Gly Gly His Glu Asp Pro Ser Phe Asn Val Pro Gly Asp		
	420	425
Asp Gln His Trp Leu Gly Ala Ser Leu Ser Arg Val Lys Asn Met Met		
435	440	445

Ala Arg Asp Lys Asn His Ala Ser Ile Leu Ile Trp Ser Leu Gly Asn
 450 455 460
 Glu Ser Tyr Ala Gly Thr Val Phe Ala Gln Met Ala Asp Tyr Val Arg
 465 470 475 480
 Lys Ala Asp Pro Thr Arg Val Gln His Tyr Glu Gly Val Thr His Asn
 485 490 495
 Arg Lys Phe Asp Asp Ala Thr Gln Ile Glu Ser Arg Met Tyr Ala Pro
 500 505 510
 Ala Lys Val Ile Glu Glu Tyr Leu Thr Asn Lys Pro Ala Lys Pro Phe
 515 520 525
 Ile Ser Val Glu Tyr Ala His Ala Met Gly Asn Ser Val Gly Asp Leu
 530 535 540
 Ala Ala Tyr Thr Ala Leu Glu Lys Tyr Pro His Tyr Gln Gly Gly Phe
 545 550 555 560
 Ile Trp Asp Trp Ile Asp Gln Gly Leu Glu Lys Asp Gly His Leu Leu
 565 570 575
 Tyr Gly Gly Asp Phe Asp Asp Arg Pro Thr Asp Tyr Glu Phe Cys Gly
 580 585 590
 Asn Gly Leu Val Phe Ala Asp Arg Thr Glu Ser Pro Lys Leu Ala Asn
 595 600 605
 Val Lys Ala Leu Tyr Ala Asn Leu Lys Leu Glu Val Lys Asp Gly Gln
 610 615 620
 Leu Phe Leu Lys Asn Asp Asn Leu Phe Thr Asn Ser Ser Ser Tyr Tyr
 625 630 635 640
 Phe Leu Thr Ser Leu Leu Val Asp Gly Lys Leu Thr Tyr Gln Ser Arg
 645 650 655
 Pro Leu Thr Phe Gly Leu Glu Pro Gly Glu Ser Gly Thr Phe Ala Leu
 660 665 670
 Pro Trp Pro Glu Val Ala Asp Glu Lys Gly Glu Val Val Tyr Arg Val
 675 680 685
 Thr Ala His Leu Lys Glu Asp Leu Pro Trp Ala Asp Glu Gly Phe Thr
 690 695 700
 Val Ala Glu Ala Glu Glu Val Ala Gln Lys Leu Pro Glu Phe Lys Pro
 705 710 715 720
 Glu Gly Arg Pro Asp Leu Val Asp Ser Asp Tyr Asn Leu Gly Leu Lys
 725 730 735
 Gly Asn Asn Phe Gln Ile Leu Phe Ser Lys Val Lys Gly Trp Pro Val
 740 745 750
 Ser Leu Lys Tyr Ala Gly Arg Glu Tyr Leu Lys Arg Leu Pro Glu Phe

755	760	765
Thr Phe Trp Arg Ala Leu	Thr Asp Asn Asp Arg Gly	Ala Gly Tyr Gly
770	775	780
Tyr Asp Leu Ala Arg Trp	Glu Asn Ala Gly Lys Tyr	Ala Arg Leu Lys
785	790	795
Asp Ile Ser Cys Glu Val	Lys Glu Asp Ser Val Leu	Val Lys Thr Ala
805	810	815
Phe Thr Leu Pro Val Ala	Leu Lys Gly Asp Leu Thr	Val Thr Tyr Glu
820	825	830
Val Asp Gly Arg Gly Lys	Ile Ala Val Thr Ala Asp	Phe Pro Gly Ala
835	840	845
Glu Glu Ala Gly Leu Leu	Pro Ala Phe Gly Leu Asn	Leu Ala Leu Pro
850	855	860
Lys Glu Leu Thr Asp Tyr	Arg Tyr Tyr Gly Leu Gly	Pro Asn Glu Ser
865	870	875
Tyr Pro Asp Arg Leu Glu	Gly Asn Tyr Leu Gly Ile	Tyr Gln Gly Ala
885	890	895
Val Lys Lys Asn Phe Ser	Pro Tyr Leu Arg Pro Gln	Glu Thr Gly Asn
900	905	910
Arg Ser Lys Val Arg Trp	Tyr Gln Leu Phe Asp Glu	Lys Gly Gly Leu
915	920	925
Glu Phe Thr Ala Asn Gly	Ala Asp Leu Asn Leu Ser	Ala Leu Pro Tyr
930	935	940
Ser Ala Ala Gln Ile Glu	Ala Ala Asp His Ala Phe	Glu Leu Thr Asn
945	950	955
Asn Tyr Thr Trp Val Arg	Ala Leu Ser Ala Gln Met	Gly Val Gly Gly
965	970	975
Asp Asp Ser Trp Gly Gln	Lys Val His Pro Glu Phe	Cys Leu Asp Ala
980	985	990
Gln Lys Ala Arg Gln Leu	Arg Leu Val Ile Gln Pro	Leu Leu Leu Lys
995	1000	1005
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<212> PRT		
<213> 德氏乳杆菌乳酸亚种		
<400> 19		
Met Ser Asn Lys Leu Val	Lys Glu Lys Arg Val Asp	Gln Ala Asp Leu
1	5	10
Ala Trp Leu Thr Asp Pro	Glu Val Tyr Glu Val Asn	Thr Ile Pro Pro

Met Tyr Leu Asn Gly Gln Arg Ile Val Phe Lys Gly Val Asn Arg His		
	340	350
Glu Phe Asp Ser Lys Leu Gly Arg Ala Ile Thr Glu Ala Asp Met Ile		
	355	365
Trp Asp Ile Lys Thr Met Lys Gln Ser Asn Ile Asn Ala Val Arg Cys		
	370	380
Ser His Tyr Pro Asn Gln Ser Leu Phe Tyr Arg Leu Cys Asp Lys Tyr		
385	390	400
Gly Leu Tyr Val Ile Asp Glu Ala Asn Leu Glu Ser His Gly Thr Trp		
	405	415
Glu Lys Val Gly His Glu Asp Pro Ser Phe Asn Val Pro Gly Asp Asp		
	420	430
Gln His Trp Leu Gly Ala Ser Leu Ser Arg Val Lys Asn Met Met Ala		
	435	445
Arg Asp Lys Asn His Ala Ser Ile Leu Ile Trp Ser Leu Gly Asn Glu		
	450	460
Ser Tyr Ala Gly Thr Val Phe Ala Gln Met Ala Asp Tyr Val Arg Lys		
465	470	480
Ala Asp Pro Thr Arg Val Gln His Tyr Glu Gly Val Thr His Asn Arg		
	485	495
Lys Phe Asp Asp Ala Thr Gln Ile Glu Ser Arg Met Tyr Ala Pro Ala		
	500	510
Lys Glu Ile Glu Glu Tyr Leu Thr Lys Lys Pro Ala Lys Pro Phe Ile		
	515	525
Ser Val Glu Tyr Ala His Ala Met Gly Asn Ser Val Gly Asp Leu Ala		
	530	540
Ala Tyr Thr Ala Leu Glu Lys Tyr Pro His Tyr Gln Gly Gly Phe Ile		
545	550	560
Trp Asp Trp Ile Asp Gln Gly Leu Glu Lys Asp Gly His Leu Leu Tyr		
	565	575
Gly Gly Asp Phe Asp Asp Arg Pro Thr Asp Tyr Glu Phe Cys Gly Asp		
	580	590
Gly Leu Val Phe Ala Asp Arg Thr Thr Ser Pro Lys Leu Ala Asn Val		
	595	605
Lys Ala Leu Tyr Ser Asn Leu Lys Leu Glu Val Lys Asp Gly Gln Leu		
	610	620
Phe Ile Lys Asn Asp Asn Leu Phe Thr Asn Ser Ser Ala Tyr Tyr Phe		
625	630	640
Leu Thr Ser Leu Leu Val Asp Gly Lys Leu Thr Tyr Gln Ser Gln Pro		

	645	650	655
Leu Thr Phe Gly	Leu Glu Pro Gly	Glu Ser Gly Thr	Phe Ala Leu Pro
	660	665	670
Trp Pro Glu Val	Glu Asp Glu Lys	Gly Glu Ile Val	Tyr Gln Val Thr
	675	680	685
Ala His Leu Lys	Glu Asp Leu Pro	Trp Ala Asp Glu	Gly Phe Thr Val
	690	695	700
Ala Glu Ala Glu	Glu Ala Val Thr	Lys Leu Pro Glu	Phe Tyr Pro Ala
705	710	715	720
Gly Arg Pro Glu	Leu Val Asp Ser	Asp Phe Asn Leu	Gly Leu Lys Gly
	725	730	735
Asn Gly Phe Arg	Ile Leu Phe Ser	Lys Ala Lys Gly	Trp Pro Val Ser
	740	745	750
Ile Lys Tyr Ala	Gly Arg Glu Tyr	Leu Lys Arg Leu	Pro Glu Phe Thr
	755	760	765
Phe Trp Arg Ala	Leu Thr Asp Asn	Asp Arg Gly Ala	Gly Tyr Gly Tyr
	770	775	780
Asp Leu Ala Lys	Trp Glu Asn Ala	Gly Lys Tyr Ala	Arg Leu Gln Asp
785	790	795	800
Ile Ser Tyr Glu	Ile Lys Glu Asn	Ser Ala Leu Val	Lys Thr Ala Phe
	805	810	815
Thr Leu Pro Val	Ala Leu Lys Gly	Asp Leu Thr Ile	Thr Tyr Glu Val
	820	825	830
Asp Ser Leu Gly	Lys Ile Ala Val	Thr Ala Asn Phe	Pro Gly Ala Val
	835	840	845
Glu Asn Gly Leu	Leu Pro Ala Phe	Gly Leu Asn Phe	Ala Leu Pro Lys
	850	855	860
Glu Leu Ser Asp	Tyr Arg Tyr Tyr	Gly Leu Gly Pro	Asn Glu Ser Tyr
865	870	875	880
Ala Asp Arg Leu	Glu Gly Ser Tyr	Leu Gly Ile Tyr	Gln Gly Met Val
	885	890	895
Glu Lys Asn Phe	Thr Pro Tyr Leu	Arg Pro Gln Glu	Ala Gly Asn Arg
	900	905	910
Ser Lys Val Arg	Tyr Tyr Gln Leu	Phe Asp Glu Glu	Gly Gly Leu Glu
	915	920	925
Phe Thr Ala Asn	Gly Ala Asp Leu	Asn Leu Ser Ala	Leu Pro Tyr Ser
	930	935	940
Ala Ala Gln Ile	Glu Ala Ala Asp	His Ala Phe Glu	Leu Thr Asn Asn
945	950	955	960

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

	165		170		175
Tyr	Glu Gly Leu Ser Gly Glu Thr	Tyr	Pro Asp Arg Met Ala Gly Ala		
	180		185		190
Lys	Glu Gly Lys Phe His Ile Asp Gly Leu Pro Val Thr Glu Tyr Leu				
	195		200		205
Val	Pro Gln Glu Asn Gly Met His Met Gln Thr Lys Lys Leu Thr Ile				
	210		215		220
Asn	Arg Glu Thr Thr Gln Asn Asn Val Asp Arg Thr Asn Glu Lys Phe				
225		230		235	240
Ser	Leu Ser Ile Gln Gln Ala Glu Lys Pro Phe Asn Phe Ser Cys Leu				
	245		250		255
Pro	Tyr Thr Ala Glu Glu Leu Glu Asn Ala Thr His Ile Glu Glu Leu				
	260		265		270
Pro	Leu Val Arg Arg Thr Val Leu Val Ile Ala Gly Ala Val Arg Gly				
	275		280		285
Val	Gly Gly Ile Asp Ser Trp Gly Thr Asp Val Glu Ser Ala Tyr His				
	290		295		300
Ile	Asn Pro Glu Leu Asp His Glu Phe Ser Phe Ile Leu Asn				
305		310		315	
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<212>	PRT				
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<400>	22				
Met	Thr Asp Val Thr His Val Asp Arg Ala Ser Gln Ala Trp Leu Thr				
1		5		10	
Asp	Pro Thr Val Phe Glu Val Asn Arg Thr Pro Ala His Ser Ser His				
	20		25		30
Lys	Trp Tyr Ala Arg Asp Pro Gln Ser Gly Gln Trp Ser Asp Leu Lys				
	35		40		45
Gln	Ser Leu Asp Gly Glu Trp Arg Val Glu Val Val Gln Ala Ala Asp				
	50		55		60
Ile	Asn Leu Glu Glu Glu Pro Ala Thr Ala Glu Ser Phe Asp Asp Ser				
65		70		75	80
Ser	Phe Glu Arg Ile Gln Val Pro Gly His Leu Gln Thr Ala Gly Leu				
	85		90		95
Met	Asn His Lys Tyr Val Asn Val Gln Tyr Pro Trp Asp Gly His Glu				
	100		105		110
Asn	Pro Leu Glu Pro Asn Ile Pro Glu Asn Asn His Val Ala Leu Tyr				

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

Val Pro Gly Ser Lys Arg Glu Trp Glu Gly Ala Cys Val Asp Arg Val
 435 440 445
 Asn Ser Met Met Arg Arg Asp Tyr Asn His Pro Ser Val Leu Ile Trp
 450 455 460
 Ser Leu Gly Asn Glu Ser Tyr Val Gly Asp Val Phe Arg Ala Met Tyr
 465 470 475 480
 Lys His Val His Asp Ile Asp Pro Asn Arg Pro Val His Tyr Glu Gly
 485 490 495
 Val Thr His Asn Arg Asp Tyr Asp Asp Val Thr Asp Ile Glu Thr Arg
 500 505 510
 Met Tyr Ser His Ala Asp Glu Ile Glu Lys Tyr Leu Lys Asp Asp Pro
 515 520 525
 Lys Lys Pro Tyr Leu Ser Cys Glu Tyr Met His Ala Met Gly Asn Ser
 530 535 540
 Val Gly Asn Met Asp Glu Tyr Thr Ala Leu Glu Arg Tyr Pro Lys Tyr
 545 550 555 560
 Gln Gly Gly Phe Ile Trp Asp Phe Ile Asp Gln Ala Ile Tyr Ala Thr
 565 570 575
 Gln Pro Asp Gly Thr Arg Ser Leu Arg Tyr Gly Gly Asp Phe Gly Asp
 580 585 590
 Arg Pro Ser Asp Tyr Glu Phe Ser Gly Asp Gly Leu Leu Phe Ala Asn
 595 600 605
 Arg Lys Pro Ser Pro Lys Ala Gln Glu Val Lys Gln Leu Tyr Ser Asn
 610 615 620
 Val His Ile Asp Val Thr Lys Asp Ser Val Ser Val Lys Asn Asp Asn
 625 630 635 640
 Leu Phe Thr Ala Thr Gly Asp Tyr Val Phe Val Leu Ser Val Leu Ala
 645 650 655
 Asp Gly Lys Pro Val Trp Gln Ser Thr Arg Arg Phe Asp Val Pro Ala
 660 665 670
 Gly Glu Thr Arg Thr Phe Asp Val Ala Trp Pro Val Ala Ala Tyr Arg
 675 680 685
 Ala Asp Ala Arg Glu Leu Val Leu Gln Val Ser Gln Arg Leu Ala Lys
 690 695 700
 Ala Thr Asp Trp Ala Glu Ser Gly Tyr Glu Leu Ala Phe Gly Gln Thr
 705 710 715 720
 Val Val Pro Ala Asp Ala Thr Ala Thr Pro Asp Thr Lys Pro Ala Asp
 725 730 735
 Gly Thr Ile Thr Val Gly Arg Trp Asn Ala Gly Val Arg Gly Ala Gly

740	745	750
Arg Glu Val Leu Leu Ser Arg Thr Gln Gly Gly Met Val Ser Tyr Thr		
755	760	765
Phe Ala Gly Asn Glu Phe Val Leu Arg Arg Pro Ala Ile Thr Thr Phe		
770	775	780
Arg Pro Leu Thr Asp Asn Asp Arg Gly Ala Gly His Gly Phe Glu Arg		
785	790	795
Val Gln Trp Leu Gly Ala Gly Arg Tyr Ala Arg Cys Val Asp Asn Val		
805	810	815
Leu Glu Gln Ile Asp Asp Ser Thr Leu Lys Gly Thr Tyr Thr Tyr Glu		
820	825	830
Leu Ala Thr Ala Gln Arg Thr Lys Val Thr Val Ser Tyr Thr Ala His		
835	840	845
Thr Asp Gly Arg Val Asn Leu His Val Glu Tyr Pro Gly Glu Gln Gly		
850	855	860
Asp Leu Pro Thr Ile Pro Ala Phe Gly Ile Glu Trp Thr Leu Pro Val		
865	870	875
Gln Tyr Thr Asn Leu Arg Phe Phe Gly Thr Gly Pro Ala Glu Thr Tyr		
885	890	895
Leu Asp Arg Lys His Ala Lys Leu Gly Val Trp Ser Thr Asn Ala Phe		
900	905	910
Ala Asp His Ala Pro Tyr Leu Met Pro Gln Glu Thr Gly Asn His Glu		
915	920	925
Asp Val Arg Trp Ala Glu Ile Thr Asp Asp His Gly His Gly Met Arg		
930	935	940
Val Ser Arg Ala Asp Gly Ala Ala Pro Phe Ala Val Ser Leu Leu Pro		
945	950	955
Tyr Ser Ser Phe Met Leu Glu Glu Ala Gln His Gln Asp Glu Leu Pro		
965	970	975
Lys Pro Lys His Met Phe Leu Arg Val Leu Ala Ala Gln Met Gly Val		
980	985	990
Gly Gly Asp Asp Ser Trp Met Ser Pro Val His Pro Gln Tyr His Ile		
995	1000	1005
Pro Ala Asp Lys Pro Ile Ser Leu Asp Val Asp Leu Glu Leu Ile		
1010	1015	1020
<210> 23		
<211> 628		
<212> PRT		
<213> 罗伊氏乳杆菌 (Lactobacillus reuteri)		

<400> 23

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

610	615	620
Gly Leu His Lys		
625		
<210> 24		
<211> 319		
<212> PRT		
<213> 罗伊氏乳杆菌		
<400> 24		
Met Ala Tyr Thr Asn Lys Leu Arg Val Ile Tyr Gly Asp Ala Thr Leu		
1	5	10
Gly Leu Ser Gly Asp Gly Phe His Tyr Ile Phe Ser Tyr Glu Arg Gly		
	20	25
Gly Leu Glu Ser Leu Lys Leu Asn Gly Lys Glu Trp Leu Tyr Arg Glu		
	35	40
Pro Met Pro Thr Phe Trp Arg Ala Thr Thr Asp Asn Asp Arg Gly Ser		
	50	55
Gly Phe Asn Ile Arg Ser Ala Gln Trp Leu Ala Ala Asp Thr Phe His		
65	70	75
Lys Cys Val Gly Ile Asp Leu Thr Val Asp Asn Gln His Phe Ala Glu		
	85	90
Leu Pro Ile Ala Pro Ile Thr Asn Glu Phe Ser Asp Pro Val Ser Ala		
	100	105
Glu Ser Val Lys Ile Lys Tyr Thr Phe Ala Thr Leu Thr Val Pro Ala		
	115	120
Thr Gln Val Thr Val Ile Tyr Glu Val Asn Gly Gln Gly Glu Ile Lys		
	130	135
Val Thr Met His Tyr Tyr Gly His Glu Asp Leu Pro Gly Leu Pro Val		
145	150	155
Val Gly Met Arg Phe Ile Met Pro Thr Val Ala Thr Gly Phe Asp Tyr		
	165	170
Gln Gly Leu Ser Gly Glu Thr Tyr Pro Asp Arg Met Ala Gly Ala Thr		
	180	185
Glu Gly Thr Phe His Val Asp Gly Leu Pro Val Thr Lys Tyr Leu Val		
	195	200
Pro Gln Glu Asn Gly Met His Met Ala Thr His Ala Leu Thr Ile Thr		
	210	215
Arg Asp Ser Thr Gln Asn Asn Ala Asp His Ser Arg Glu Pro Phe Ser		
225	230	235
Leu Thr Val Lys Gln Asp Ala Gln Pro Phe Ala Phe Ser Cys Leu Pro		

	245	250	255
Tyr Thr Ala Glu Glu Leu Glu Asn Ala Thr His Ile Glu Glu Leu Pro			
	260	265	270
Leu Ala Arg Arg Thr Val Leu Val Val Ala Gly Ala Val Arg Gly Val			
	275	280	285
Gly Gly Ile Asp Ser Trp Gly Ala Asp Val Glu Glu Gln Tyr His Ile			
	290	295	300
Pro Ala Asp Arg Asp Val Glu Phe Ser Phe Val Leu Asn Ala Lys			
305	310	315	
<210> 25			
<211> 1007			
<212> PRT			
<213> 德氏乳杆菌乳酸亚种			
<400> 25			
Met Ser Asn Lys Leu Val Lys Glu Lys Arg Val Asp Gln Ala Asp Leu			
1	5	10	15
Ala Trp Leu Thr Asp Pro Glu Val Tyr Glu Val Asn Thr Ile Pro Pro			
	20	25	30
His Ser Asp His Glu Ser Phe Gln Ser Gln Glu Glu Leu Glu Glu Gly			
	35	40	45
Lys Ser Ser Leu Val Gln Ser Leu Asp Gly Asn Trp Leu Ile Asp Tyr			
	50	55	60
Ala Glu Asn Gly Gln Gly Pro Ile Asn Phe Tyr Ala Glu Asp Phe Asp			
65	70	75	80
Asp Ser Asn Phe Lys Ser Val Lys Val Pro Gly Asn Leu Glu Leu Gln			
	85	90	95
Gly Phe Gly Gln Pro Gln Tyr Val Asn Ile Gln Tyr Pro Trp Asp Gly			
	100	105	110
Ser Glu Glu Ile Phe Pro Pro Gln Val Pro Ser Lys Ile Pro Leu Ala			
	115	120	125
Ser Tyr Val Arg Tyr Phe Asp Leu Asp Glu Ala Leu Trp Asp Lys Glu			
	130	135	140
Val Ser Leu Lys Phe Ala Gly Ala Ala Thr Ala Ile Tyr Val Trp Leu			
145	150	155	160
Asn Gly His Phe Val Gly Tyr Gly Glu Asp Ser Phe Thr Pro Ser Glu			
	165	170	175
Phe Met Val Thr Lys Phe Leu Lys Lys Glu Gly Asn Arg Leu Ala Val			
	180	185	190
Ala Leu Tyr Lys Tyr Ser Ser Ala Ser Trp Leu Glu Asp Gln Asp Phe			

195	200	205
Trp Arg Leu Ser Gly Leu Phe Arg Ser Val Thr Leu Glu Ala Lys Pro		
210	215	220
Leu Leu His Leu Glu Asp Leu Lys Leu Thr Ala Ser Leu Thr Asp Asn		
225	230	235
240		
Tyr Gln Lys Gly Lys Leu Glu Val Glu Ala Asn Ile Ala Tyr Arg Leu		
245	250	255
Pro Asn Ala Ser Phe Lys Leu Glu Val Arg Asp Ser Glu Gly Asp Leu		
260	265	270
Val Ala Glu Lys Val Gly Pro Ile Arg Ser Glu Lys Leu Asp Phe Ser		
275	280	285
Leu Ala Asp Leu Pro Val Ala Ala Trp Ser Ala Glu Lys Pro Asn Leu		
290	295	300
Tyr Gln Val Arg Leu Tyr Leu Tyr Gln Ala Gly Ser Leu Leu Glu Val		
305	310	315
320		
Ser Arg Gln Glu Val Gly Phe Arg Asn Phe Glu Leu Lys Asp Gly Ile		
325	330	335
Met Tyr Leu Asn Gly Gln Arg Ile Val Phe Lys Gly Val Asn Arg His		
340	345	350
Glu Phe Asp Ser Lys Leu Gly Arg Ala Ile Thr Glu Ala Asp Met Ile		
355	360	365
Trp Asp Ile Lys Thr Met Lys Gln Ser Asn Ile Asn Ala Val Arg Cys		
370	375	380
Ser His Tyr Pro Asn Gln Ser Leu Phe Tyr Arg Leu Cys Asp Lys Tyr		
385	390	395
400		
Gly Leu Tyr Val Ile Asp Glu Ala Asn Leu Glu Ser His Gly Thr Trp		
405	410	415
Glu Lys Val Gly His Glu Asp Pro Ser Phe Asn Val Pro Gly Asp Asp		
420	425	430
Gln His Trp Leu Gly Ala Ser Leu Ser Arg Val Lys Asn Met Met Ala		
435	440	445
Arg Asp Lys Asn His Ala Ser Ile Leu Ile Trp Ser Leu Gly Asn Glu		
450	455	460
Ser Tyr Ala Gly Thr Val Phe Ala Gln Met Ala Asp Tyr Val Arg Lys		
465	470	475
480		
Ala Asp Pro Thr Arg Val Gln His Tyr Glu Gly Val Thr His Asn Arg		
485	490	495
Lys Phe Asp Asp Ala Thr Gln Ile Glu Ser Arg Met Tyr Ala Pro Ala		
500	505	510

Lys Glu Ile Glu Glu Tyr Leu Thr Lys Lys Pro Ala Lys Pro Phe Ile
 515 520 525
 Ser Val Glu Tyr Ala His Ala Met Gly Asn Ser Val Gly Asp Leu Ala
 530 535 540
 Ala Tyr Thr Ala Leu Glu Lys Tyr Pro His Tyr Gln Gly Gly Phe Ile
 545 550 555 560
 Trp Asp Trp Ile Asp Gln Gly Leu Glu Lys Asp Gly His Leu Leu Tyr
 565 570 575
 Gly Gly Asp Phe Asp Asp Arg Pro Thr Asp Tyr Glu Phe Cys Gly Asp
 580 585 590
 Gly Leu Val Phe Ala Asp Arg Thr Thr Ser Pro Lys Leu Ala Asn Val
 595 600 605
 Lys Ala Leu Tyr Ser Asn Leu Lys Leu Glu Val Lys Asp Gly Gln Leu
 610 615 620
 Phe Ile Lys Asn Asp Asn Leu Phe Thr Asn Ser Ser Ala Tyr Tyr Phe
 625 630 635 640
 Leu Thr Ser Leu Leu Val Asp Gly Lys Leu Thr Tyr Gln Ser Gln Pro
 645 650 655
 Leu Thr Phe Gly Leu Glu Pro Gly Glu Ser Gly Thr Phe Ala Leu Pro
 660 665 670
 Trp Pro Glu Val Glu Asp Glu Lys Gly Glu Ile Val Tyr Gln Val Thr
 675 680 685
 Ala His Leu Lys Glu Asp Leu Pro Trp Ala Asp Glu Gly Phe Thr Val
 690 695 700
 Ala Glu Ala Glu Glu Ala Val Thr Lys Leu Pro Glu Phe Tyr Pro Ala
 705 710 715 720
 Gly Arg Pro Glu Leu Val Asp Ser Asp Phe Asn Leu Gly Leu Lys Gly
 725 730 735
 Asn Gly Phe Arg Ile Leu Phe Ser Lys Ala Lys Gly Trp Pro Val Ser
 740 745 750
 Ile Lys Tyr Ala Gly Arg Glu Tyr Leu Lys Arg Leu Pro Glu Phe Thr
 755 760 765
 Phe Trp Arg Ala Leu Thr Asp Asn Asp Arg Gly Ala Gly Tyr Gly Tyr
 770 775 780
 Asp Leu Ala Lys Trp Glu Asn Ala Gly Lys Tyr Ala Arg Leu Gln Asp
 785 790 795 800
 Ile Ser Tyr Glu Ile Lys Glu Asn Ser Ala Leu Val Lys Thr Thr Phe
 805 810 815
 Thr Leu Pro Val Ala Leu Lys Gly Asp Leu Thr Ile Thr Tyr Glu Val

820	825	830
Asp Ser Leu Gly Lys Ile Ala Val Thr Ala Asn Phe Pro Gly Ala Val		
835	840	845
Glu Asn Gly Leu Leu Pro Ala Phe Gly Leu Asn Phe Ala Leu Pro Lys		
850	855	860
Glu Leu Ser Asp Tyr Arg Tyr Tyr Gly Leu Gly Pro Asn Glu Ser Tyr		
865	870	875
Ala Asp Arg Leu Glu Gly Ser Tyr Leu Gly Ile Tyr Gln Gly Met Val		
885	890	895
Glu Lys Asn Phe Thr Pro Tyr Leu Arg Pro Gln Glu Ala Gly Asn Arg		
900	905	910
Ser Lys Val Arg Tyr Tyr Gln Leu Phe Asp Glu Glu Gly Gly Leu Glu		
915	920	925
Phe Thr Ala Asn Gly Ala Asp Leu Asn Leu Ser Ala Leu Pro Tyr Ser		
930	935	940
Ala Ala Gln Ile Glu Ala Ala Asp His Ala Phe Glu Leu Thr Asn Asn		
945	950	955
Tyr Thr Trp Val Arg Ala Leu Ala Ala Gln Met Gly Val Gly Gly Asp		
965	970	975
Asp Ser Trp Gly Gln Lys Val His Pro Glu Phe Cys Leu Asp Ala Gln		
980	985	990
Glu Ala Arg Gln Leu Lys Leu Val Ile Gln Pro Leu Leu Leu Lys		
995	1000	1005
<210> 26		
<211> 628		
<212> PRT		
<213> 瑞士乳杆菌		
<400> 26		
Met Gln Ala Asn Ile Asn Trp Leu Asp Asn Pro Glu Val Phe Arg Val		
1	5	10
Asn Gln Leu Pro Ala His Ser Asp His Pro Phe Phe Arg Asp Tyr Arg		
20	25	30
Glu Trp Gln Lys Gln His Ser Ser Tyr Gln Gln Ser Leu Asn Gly Lys		
35	40	45
Trp Lys Phe His Phe Ser Ala Asn Pro Met Asp Arg Pro Gln Asp Phe		
50	55	60
Tyr Gln Arg Asp Phe Asp Ser Ser Asn Phe Asp Ser Ile Pro Val Pro		
65	70	75
Ser Glu Ile Glu Leu Ser Asn Tyr Thr Gln Asn Gln Tyr Ile Asn Val		

	85	90	95
Leu Phe Pro Trp	Glu Gly Lys Ile	Phe Arg Arg Pro	Ala Tyr Ala Leu
	100	105	110
Asp Pro Asn Asp	His Glu Glu Gly	Ser Phe Ser Lys	Gly Ala Asp Asn
	115	120	125
Thr Val Gly Ser	Tyr Leu Lys Arg	Phe Asp Leu Ser	Ser Ala Leu Ile
	130	135	140
Gly Lys Asp Val	His Ile Lys Phe	Glu Gly Val Glu	Gln Ala Met Tyr
145	150	155	160
Val Trp Leu Asn	Gly His Phe Val	Gly Tyr Ala Glu	Asp Ser Phe Thr
	165	170	175
Pro Ser Glu Phe	Asp Leu Thr Pro	Tyr Ile Gln Glu	Lys Asp Asn Leu
	180	185	190
Leu Ala Val Glu	Val Phe Lys His	Ser Thr Ala Ser	Trp Leu Glu Asp
	195	200	205
Gln Asp Met Phe	Arg Phe Ser Gly	Ile Phe Arg Ser	Val Glu Leu Leu
210	215	220	
Gly Ile Pro Ala	Thr His Leu Met	Asp Met Asp Leu	Lys Pro Arg Val
225	230	235	240
Ala Asp Asn Tyr	Gln Asp Gly Ile	Phe Asn Leu Lys	Leu His Phe Ile
	245	250	255
Gly Lys Lys Ala	Gly Ser Phe His	Leu Leu Val Lys	Asp Ile Lys Gly
	260	265	270
His Thr Leu Leu	Glu Lys Asn Glu	Asp Ile Lys Glu	Asn Val Gln Ile
	275	280	285
Asn Asn Glu Lys	Phe Glu Asn Val	His Leu Trp Asn	Asn His Asp Pro
	290	295	300
Tyr Leu Tyr Gln	Leu Leu Ile Glu	Val Tyr Asp Glu	Gln Gln Asn Leu
305	310	315	320
Leu Glu Leu Ile	Pro Phe Gln Phe	Gly Phe Arg Arg	Ile Glu Ile Ser
	325	330	335
Pro Glu Lys Val	Val Leu Leu Asn	Gly Lys Arg Leu	Ile Ile Asn Gly
	340	345	350
Val Asn Arg His	Glu Trp Asp Ala	Lys Arg Gly Arg	Ser Ile Thr Met
	355	360	365
Ser Asp Met Thr	Thr Asp Ile Asn	Thr Phe Lys Glu	Asn Asn Ile Asn
	370	375	380
Ala Val Arg Thr	Cys His Tyr Pro	Asn Gln Ile Pro	Trp Tyr Tyr Leu
385	390	395	400

Cys Asp Gln Asn Gly Ile Tyr Val Met Ala Glu Asn Asn Leu Glu Ser
 405 410 415
 His Gly Thr Trp Gln Lys Met Gly Glu Ile Glu Pro Ser Asp Asn Val
 420 425 430
 Pro Gly Ser Ile Pro Gln Trp Lys Glu Ala Val Ile Asp Arg Ala Arg
 435 440 445
 Asn Asn Tyr Glu Thr Phe Lys Asn His Thr Ser Ile Leu Phe Trp Ser
 450 455 460
 Leu Gly Asn Glu Ser Tyr Ala Gly Asp Asn Ile Ile Ala Met Asn Glu
 465 470 475 480
 Phe Tyr Lys Ser His Asp Asp Thr Arg Leu Val His Tyr Glu Gly Val
 485 490 495
 Val His Arg Pro Glu Leu Lys Asp Lys Ile Ser Asp Val Glu Ser Cys
 500 505 510
 Met Tyr Leu Pro Pro Lys Lys Val Glu Glu Tyr Leu Gln Asn Asp Pro
 515 520 525
 Pro Lys Pro Phe Met Glu Cys Glu Tyr Met His Asp Met Gly Asn Ser
 530 535 540
 Asn Gly Gly Met Asp Ser Tyr Ile Lys Leu Leu Asp Lys Tyr Pro Gln
 545 550 555 560
 Tyr Phe Gly Gly Phe Ile Trp Asp Phe Ile Asp Gln Ala Leu Leu Val
 565 570 575
 His Asp Glu Ile Ser Gly His Asp Val Leu Arg Tyr Gly Gly Asp Phe
 580 585 590
 Asp Asp Arg His Ser Asp Tyr Glu Phe Ser Gly Asp Gly Leu Met Phe
 595 600 605
 Ala Asp Arg Lys Pro Lys Pro Ala Met Gln Glu Val Arg Tyr Tyr Tyr
 610 615 620
 Gly Leu His Lys
 625
 <210> 27
 <211> 318
 <212> PRT
 <213> 瑞士乳杆菌
 <400> 27
 Met Asp Tyr Thr Asn Asn Gln Leu His Ile Ile Tyr Gly Asp Ala Thr
 1 5 10 15
 Phe Gly Val Asn Gly Lys Asp Phe Gln Tyr Ile Phe Ser Tyr Glu Arg
 20 25 30

Gly Gly Leu Glu Ser Leu Lys Val His Gly Lys Glu Trp Leu Tyr Arg
 35 40 45
 Val Pro Thr Pro Thr Phe Trp Arg Ala Thr Thr Asp Asn Asp Arg Gly
 50 55 60
 Ser Gly Phe Asn Leu Lys Ala Ala Gln Trp Leu Gly Ala Asp Met Phe
 65 70 75 80
 Thr Lys Cys Thr Asp Ile His Leu Lys Val Asp Arg His Asp Phe Ala
 85 90 95
 Glu Leu Pro Ile Ala Pro Phe Asn Asn Lys Phe Ser Asn His Glu Tyr
 100 105 110
 Ala Lys Ser Ala Glu Ile Ser Phe Thr Tyr Gln Thr Leu Thr Thr Pro
 115 120 125
 Ala Thr Asn Ala Lys Ile Ile Tyr Asn Ile Asp Asp Gly Gly His Ile
 130 135 140
 Lys Val Thr Met Arg Tyr Tyr Gly Lys Lys Gly Leu Pro Pro Leu Pro
 145 150 155 160
 Val Ile Gly Ile Arg Leu Ile Met Pro Thr Ala Ala Thr Gly Phe Asp
 165 170 175
 Tyr Glu Gly Leu Ser Gly Glu Thr Tyr Pro Asp Arg Met Ala Gly Ala
 180 185 190
 Lys Glu Gly Lys Phe His Ile Asp Gly Leu Pro Val Thr Glu Tyr Leu
 195 200 205
 Val Pro Gln Glu Asn Gly Met His Met Gln Thr Lys Lys Leu Thr Ile
 210 215 220
 Asn Arg Glu Thr Thr Gln Asn Asn Val Asp Arg Thr Asn Glu Lys Phe
 225 230 235 240
 Ser Leu Ser Ile Gln Gln Ala Glu Lys Pro Phe Asn Phe Ser Cys Leu
 245 250 255
 Pro Tyr Thr Ala Glu Glu Leu Glu Asn Ala Thr His Ile Glu Glu Leu
 260 265 270
 Pro Leu Val Arg Arg Thr Val Leu Val Ile Ala Gly Ala Val Arg Gly
 275 280 285
 Val Gly Gly Ile Asp Ser Trp Gly Thr Asp Val Glu Ser Ala Tyr His
 290 295 300
 Ile Asn Pro Asp Leu Asp His Glu Phe Ser Phe Ile Leu Asn
 305 310 315
 <210> 28
 <211> 626
 <212> PRT

<213> 卷曲乳杆菌 (*Lactobacillus crispatus*)

<400> 28

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

290	295	300
Leu Tyr Gln Leu Ile Ile Glu Val His Asp Gln Asp Gly Lys Leu Val		
305	310	315
Glu Leu Ile Pro Tyr Gln Phe Gly Phe Arg Lys Ile Glu Ile Thr Lys		
	325	330
Asp His Val Val Leu Leu Asn Gly Lys Arg Leu Ile Ile Asn Gly Val		
	340	345
Asn Arg His Glu Trp Asp Ala Lys Arg Gly Arg Ser Ile Thr Leu Ala		
	355	360
Asp Met Lys Gln Asp Ile Ala Thr Phe Lys His Asn Asn Ile Asn Ala		
370	375	380
Val Arg Thr Cys His Tyr Pro Asn Gln Ile Pro Trp Tyr Tyr Leu Cys		
385	390	395
Asp Gln Asn Gly Ile Tyr Met Met Ala Glu Asn Asn Leu Glu Ser His		
	405	410
Gly Thr Trp Gln Lys Leu Gly Gln Val Glu Ala Thr Ser Asn Val Pro		
	420	425
Gly Ser Ile Pro Glu Trp Arg Glu Val Val Val Asp Arg Ala Arg Ser		
435	440	445
Asn Tyr Glu Thr Phe Lys Asn His Thr Ala Ile Leu Phe Trp Ser Leu		
450	455	460
Gly Asn Glu Ser Tyr Ala Gly Ser Asn Ile Ala Ala Met Asn Lys Leu		
465	470	475
Tyr Lys Asp His Asp Ser Ser Arg Leu Thr His Tyr Glu Gly Val Phe		
	485	490
His Ala Pro Glu Phe Lys Lys Glu Ile Ser Asp Leu Glu Ser Cys Met		
	500	505
Tyr Leu Pro Pro Lys Glu Ala Glu Glu Tyr Leu Gln Asn Pro Lys Lys		
	515	520
Pro Leu Val Glu Cys Glu Tyr Met His Asp Met Gly Asn Ser Asp Gly		
530	535	540
Gly Ile Gly Ser Tyr Ile Lys Leu Ile Asp Lys Tyr Pro Gln Tyr Met		
545	550	555
Gly Gly Phe Ile Trp Asp Phe Ile Asp Gln Ala Leu Leu Val His Asp		
	565	570
Pro Val Ser Gly Gln Asp Val Leu Arg Tyr Gly Gly Asp Phe Asp Asp		
	580	585
Arg His Ser Asp Tyr Glu Phe Ser Gly Asp Gly Leu Met Phe Ala Asp		
595	600	605

Arg Thr Pro Lys Pro Ala Met Gln Glu Val Arg Tyr Tyr Tyr Gly Leu
 610 615 620
 His Lys
 625
 <210> 29
 <211> 316
 <212> PRT
 <213> 卷曲乳杆菌
 <400> 29
 Met Ala Tyr Thr Asn Asn Leu His Val Val Tyr Gly Glu Ala Ser Leu
 1 5 10 15
 Gly Val Asn Gly Gln Asp Phe Ala Tyr Leu Phe Ser Tyr Glu Arg Gly
 20 25 30
 Val Leu Glu Ser Leu Lys Ile Lys Asp Lys Glu Trp Leu Tyr Arg Thr
 35 40 45
 Pro Thr Pro Thr Phe Trp Arg Ala Thr Thr Asp Asn Asp Arg Gly Ser
 50 55 60
 Gly Phe Asn Gln Lys Ala Ala Gln Trp Leu Gly Ala Asp Met Phe Thr
 65 70 75 80
 Lys Cys Val Gly Ile His Val Gln Val Asp Asp His Gln Phe Asp Glu
 85 90 95
 Leu Pro Ile Ala Pro Ile Asn Asn Gln Phe Ser Asn Gln Glu Phe Ala
 100 105 110
 His Glu Val Lys Val Ala Phe Asp Tyr Glu Thr Leu Thr Thr Pro Ala
 115 120 125
 Thr Lys Val Lys Ile Ile Tyr Asn Ile Asn Asp Ala Gly His Met Thr
 130 135 140
 Ile Thr Met His Tyr Phe Gly Lys Lys Gly Leu Pro Pro Leu Pro Val
 145 150 155 160
 Ile Gly Met Arg Phe Ile Met Pro Thr Lys Ala Lys Ser Phe Asp Tyr
 165 170 175
 Thr Gly Leu Ser Gly Glu Thr Tyr Pro Asp Arg Met Ala Gly Ala Glu
 180 185 190
 Arg Gly Thr Phe His Ile Asp Gly Leu Pro Val Thr Lys Tyr Leu Val
 195 200 205
 Pro Gln Glu Asn Gly Met His Met Gln Thr Asn Glu Leu Val Ile Thr
 210 215 220
 Arg Asn Ser Thr Gln Asn Asn Ala Asp Lys Asp Gly Asp Phe Ser Leu
 225 230 235 240

Lys Ile Thr Gln Thr Lys Gln Pro Phe Asn Phe Ser Leu Leu Pro Tyr
 245 250 255
 Thr Ala Glu Glu Leu Glu Asn Ala Thr His Ile Glu Glu Leu Pro Leu
 260 265 270
 Ala Arg Arg Ser Val Leu Val Ile Ala Gly Ala Val Arg Gly Val Gly
 275 280 285
 Gly Ile Asp Ser Trp Gly Ser Asp Val Glu Glu Gln Tyr His Ile Asp
 290 295 300
 Pro Glu Gln Asp His Glu Phe Ser Phe Thr Leu Asn
 305 310 315
 <210> 30
 <211> 1025
 <212> PRT
 <213> 嗜热链球菌 (Streptococcus thermophilus)
 <400> 30
 Met Asn Met Thr Lys Ile Gln Thr Tyr Leu Asn Asp Pro Lys Ile Val
 1 5 10 15
 Ser Val Asn Thr Val Asp Ala His Ser Asp His Lys Tyr Phe Glu Ser
 20 25 30
 Leu Glu Glu Phe Ser Glu Gly Glu Met Lys Leu Arg Gln Ser Leu Asn
 35 40 45
 Gly Lys Trp Lys Ile His Tyr Ala Gln Asn Thr Asn Gln Val Leu Lys
 50 55 60
 Asp Phe Tyr Lys Thr Glu Phe Asp Glu Thr Asp Leu Asn Phe Ile Asn
 65 70 75 80
 Val Pro Gly His Leu Glu Leu Gln Gly Phe Gly Ser Pro Gln Tyr Val
 85 90 95
 Asn Thr Gln Tyr Pro Trp Asp Gly Lys Glu Phe Leu Arg Pro Pro Gln
 100 105 110
 Val Pro Gln Glu Ser Asn Ala Val Ala Ser Tyr Val Lys His Phe Thr
 115 120 125
 Leu Asn Asp Ala Leu Lys Asp Lys Lys Val Phe Ile Ser Phe Gln Gly
 130 135 140
 Val Ala Thr Ser Ile Phe Val Trp Val Asn Gly Asn Phe Val Gly Tyr
 145 150 155 160
 Ser Glu Asp Ser Phe Thr Pro Ser Glu Phe Glu Ile Ser Asp Tyr Leu
 165 170 175
 Val Glu Gly Asp Asn Lys Leu Ala Val Ala Val Tyr Arg Tyr Ser Thr
 180 185 190

Ala Ser Trp Leu Glu Asp Gln Asp Phe Trp Arg Leu Tyr Gly Ile Phe
 195 200 205
 Arg Asp Val Tyr Leu Tyr Ala Ile Pro Lys Val His Val Gln Asp Leu
 210 215 220
 Phe Val Lys Gly Asp Tyr Asp Tyr Gln Thr Lys Ala Gly Gln Leu Asp
 225 230 235 240
 Ile Asp Leu Lys Thr Val Gly Asp Tyr Glu Asp Lys Lys Ile Lys Tyr
 245 250 255
 Val Leu Ser Asp Tyr Glu Gly Ile Val Thr Glu Gly Asp Ala Ser Val
 260 265 270
 Asn Gly Asp Gly Glu Leu Ser Val Ser Leu Glu Asn Leu Lys Ile Lys
 275 280 285
 Pro Trp Ser Ala Glu Ser Pro Lys Leu Tyr Asp Leu Ile Leu His Val
 290 295 300
 Leu Asp Asp Asp Gln Val Val Glu Val Val Pro Val Lys Val Gly Phe
 305 310 315 320
 Arg Arg Phe Glu Ile Lys Asp Lys Leu Met Leu Leu Asn Gly Lys Arg
 325 330 335
 Ile Val Phe Lys Gly Val Asn Arg His Glu Phe Asn Ala Arg Thr Gly
 340 345 350
 Arg Cys Ile Thr Glu Glu Asp Met Leu Trp Asp Ile Lys Val Met Lys
 355 360 365
 Gln His Asn Ile Asn Ala Val Arg Thr Ser His Tyr Pro Asn Gln Thr
 370 375 380
 Arg Trp Tyr Glu Leu Cys Asp Glu Tyr Gly Leu Tyr Val Ile Asp Glu
 385 390 395 400
 Ala Asn Leu Glu Thr His Gly Thr Trp Gln Lys Leu Gly Leu Cys Glu
 405 410 415
 Pro Ser Trp Asn Ile Pro Ala Ser Glu Pro Glu Trp Leu Pro Ala Cys
 420 425 430
 Leu Asp Arg Ala Asn Asn Met Phe Gln Arg Asp Lys Asn His Ala Ser
 435 440 445
 Val Ile Ile Trp Ser Cys Gly Asn Glu Ser Tyr Ala Gly Lys Asp Ile
 450 455 460
 Ala Asp Met Ala Asp Tyr Phe Arg Ser Val Asp Asn Thr Arg Pro Val
 465 470 475 480
 His Tyr Glu Gly Val Ala Trp Cys Arg Glu Phe Asp Tyr Ile Thr Asp
 485 490 495
 Ile Glu Ser Arg Met Tyr Ala Lys Pro Ala Asp Ile Glu Glu Tyr Leu

Thr Asp Thr Gln Ile Gln Ile Glu Asp Asp Ser Val Lys Val Thr Tyr
 820 825 830
 Val His Glu Leu Pro Gly Leu Ser Asp Val Glu Val Lys Val Thr Tyr
 835 840 845
 Gln Val Asp Tyr Lys Gly Arg Ile Phe Val Thr Ala Asn Tyr Asp Gly
 850 855 860
 Lys Ala Gly Leu Pro Asn Phe Pro Glu Phe Gly Leu Glu Phe Ala Ile
 865 870 875 880
 Gly Ser Gln Phe Thr Asn Leu Ser Tyr Tyr Gly Tyr Gly Ala Glu Glu
 885 890 895
 Ser Tyr Arg Asp Lys Leu Pro Gly Ala Tyr Leu Gly Arg Tyr Glu Thr
 900 905 910
 Ser Val Glu Lys Thr Phe Ala Pro Tyr Leu Met Pro Gln Glu Ser Gly
 915 920 925
 Asn His Tyr Gly Thr Arg Glu Phe Thr Val Ser Asp Asp Asn His Asn
 930 935 940
 Gly Leu Lys Phe Thr Ala Leu Asn Lys Ala Phe Glu Phe Ser Ala Leu
 945 950 955 960
 Arg Asn Ser Thr Glu Gln Ile Glu Asn Ala Arg His Gln Tyr Glu Leu
 965 970 975
 Gln Glu Ser Asp Ala Thr Trp Ile Lys Val Leu Ala Ala Gln Met Gly
 980 985 990
 Val Gly Gly Asp Asp Ser Trp Gly Ala Pro Val His Asp Glu Phe Leu
 995 1000 1005
 Leu Ser Ser Ala Asp Ser Tyr Gln Leu Ser Phe Met Ile Glu Pro
 1010 1015 1020
 Leu Asn
 1025
 <210> 31
 <211> 1008
 <212> PRT
 <213> 德氏乳杆菌印度亚种 (*Lactobacillus delbrueckii* subsp. *indicus*)
 <400> 31
 Met Asn Asn Lys Leu Ala Gln Val Lys Arg Val Asp Gln Ala Asp Leu
 1 5 10 15
 Ala Trp Leu Thr Asp Pro Glu Ile Tyr Glu Val Asn Thr Ile Pro Pro
 20 25 30
 His Ser Asp His Glu Ser Phe Gln Ser Leu Glu Glu Leu Glu Glu Gly
 35 40 45

Lys Ser Ser Leu Val Gln Ser Leu Asp Gly Asp Trp Leu Ile Asp Tyr
 50 55 60
 Ala Glu Asn Gly Glu Gly Pro Ala Asn Phe Tyr Glu Glu Asn Phe Asp
 65 70 75 80
 Asp Ser Ser Phe Lys Ser Val Lys Val Pro Gly Asn Leu Glu Leu Gln
 85 90 95
 Gly Phe Gly Gln Pro Gln Tyr Val Asn Val Gln Tyr Pro Trp Asp Gly
 100 105 110
 Ser Asp Glu Ile Phe Pro Pro Met Ile Pro Ser Lys Asn Pro Val Ala
 115 120 125
 Ser Tyr Val Arg Tyr Phe Asp Leu Glu Glu Ala Phe Trp Asp Lys Glu
 130 135 140
 Val Ser Leu Lys Phe Ala Gly Ala Ala Thr Ala Ile Tyr Val Trp Leu
 145 150 155 160
 Asn Gly His Phe Val Gly Tyr Gly Glu Asp Ser Phe Thr Pro Ser Glu
 165 170 175
 Phe Met Val Thr Lys Phe Leu Lys Lys Glu Gly Asn Arg Leu Ala Val
 180 185 190
 Ala Leu Tyr Lys Tyr Ser Ser Ala Ser Trp Leu Glu Asp Gln Asp Phe
 195 200 205
 Trp Arg Met Ser Gly Leu Phe Arg Ser Val Thr Leu Glu Ala Lys Pro
 210 215 220
 Leu Leu His Leu Gln Asp Leu Lys Leu Thr Ala Ser Leu Thr Asn Asp
 225 230 235 240
 Tyr Gln Lys Gly Ser Leu Gln Val Glu Ala Asp Ile Asp Tyr Arg Leu
 245 250 255
 Pro Asn Ser Ser Phe Lys Leu Glu Leu Arg Asp Ser Ala Gly Glu Leu
 260 265 270
 Val Ala Glu Lys Val Gly Pro Ile Arg Ser Glu Lys Leu Asp Phe Ser
 275 280 285
 Leu Ala Asp Leu Pro Val Ala Ala Trp Ser Ala Glu Glu Pro Asn Leu
 290 295 300
 Tyr Gln Val Arg Leu Ser Leu Tyr Gln Gln Gly Ser Leu Leu Glu Val
 305 310 315 320
 Ser Arg Gln Glu Val Gly Phe Arg Asn Phe Glu Leu Lys Asp Gly Ile
 325 330 335
 Met Tyr Leu Asn Gly Lys Arg Ile Val Phe Lys Gly Val Asn Arg His
 340 345 350
 Glu Phe Asp Ser Lys Leu Gly Arg Ala Ile Thr Glu Ala Asp Met Ile

355	360	365
Trp Asp Ile Lys Thr Met Lys Gln Ser Asn Ile Asn Ala Val Arg Cys		
370	375	380
Ser His Tyr Pro Asn Gln Ser Ile Phe Tyr His Leu Cys Asp Lys Tyr		
385	390	395
Gly Leu Tyr Val Ile Asp Glu Ala Asn Leu Glu Ser His Gly Thr Trp		
405	410	415
Glu Lys Val Gly Gly His Glu Asp Pro Ser Phe Asn Val Pro Gly Asp		
420	425	430
Asp Gln Arg Trp Leu Gly Ala Ser Leu Ser Arg Val Lys Asn Met Met		
435	440	445
Ala Arg Asp Lys Asn His Ala Ser Ile Leu Ile Trp Ser Leu Gly Asn		
450	455	460
Glu Ser Tyr Ala Gly Lys Val Phe Ala Gln Met Ala Asp Tyr Val Arg		
465	470	475
Gln Ala Asp Pro Thr Arg Val Gln His Tyr Glu Gly Val Thr His Asn		
485	490	495
Arg Lys Phe Asp Asp Ala Thr Gln Ile Glu Ser Arg Met Tyr Ala Pro		
500	505	510
Ala Lys Glu Ile Glu Glu Tyr Leu Thr Lys Lys Pro Ala Lys Pro Phe		
515	520	525
Val Ser Cys Glu Tyr Ala His Ala Met Gly Asn Ser Val Gly Asp Leu		
530	535	540
Ala Ala Tyr Thr Ala Leu Glu Lys Tyr Pro His Tyr Gln Gly Gly Phe		
545	550	555
Ile Trp Asp Trp Ile Asp Gln Gly Leu Glu Lys Glu Gly His Leu Leu		
565	570	575
Tyr Gly Gly Asp Phe Asp Asp Arg Pro Ser Asp Tyr Glu Phe Cys Gly		
580	585	590
Asp Gly Leu Val Phe Ala Asp Arg Thr Thr Ser Pro Lys Leu Ala Asn		
595	600	605
Val Lys Ala Leu Tyr Ser Asn Leu Lys Leu Glu Leu Lys Asp Gly Gln		
610	615	620
Leu Phe Leu Lys Asn Asp Asn Leu Phe Thr Asn Ser Ser Ala Tyr Tyr		
625	630	635
Phe Leu Thr Ser Leu Leu Val Asp Gly Lys Leu Thr Tyr Gln Ser Gln		
645	650	655
Pro Leu Thr Phe Ala Leu Glu Pro Gly Glu Ser Gly Thr Phe Ala Leu		
660	665	670

Pro Trp Pro Glu Val Glu Asp Glu Lys Gly Glu Ile Val Tyr Gln Val
 675 680 685
 Thr Ala His Leu Lys Glu Asp Leu Pro Trp Ala Asp Glu Gly Phe Thr
 690 695 700
 Val Ala Glu Ala Glu Glu Ala Val Thr Lys Leu Pro Glu Phe Tyr Pro
 705 710 715 720
 Ala Gly Arg Pro Glu Leu Val Asp Ser Asp Tyr Asn Leu Gly Ile Lys
 725 730 735
 Gly Asn Gly Phe Arg Ile Leu Phe Ser Lys Ala Lys Gly Trp Pro Val
 740 745 750
 Ser Ile Lys Tyr Ala Gly Arg Glu Tyr Leu Lys Arg Leu Pro Glu Phe
 755 760 765
 Thr Phe Trp Arg Ala Leu Thr Asp Asn Asp Arg Gly Ala Gly Tyr Gly
 770 775 780
 Tyr Asp Leu Ala Lys Trp Glu Asn Ala Gly Lys Tyr Ala Arg Leu Gln
 785 790 795 800
 Asp Ile Ser Tyr Glu Ile Lys Glu Asn Ser Val Leu Val Lys Thr Ala
 805 810 815
 Phe Thr Leu Pro Val Ala Leu Lys Gly Asp Leu Thr Ile Thr Tyr Glu
 820 825 830
 Val Asp Ser Leu Gly Lys Ile Ala Val Thr Ala Asn Phe Pro Gly Ala
 835 840 845
 Val Glu Asn Gly Leu Leu Pro Ala Phe Gly Leu Asn Phe Ala Leu Pro
 850 855 860
 Lys Glu Leu Ser Asp Tyr Arg Tyr Tyr Gly Leu Gly Pro Asn Glu Ser
 865 870 875 880
 Tyr Ala Asp Arg Leu Glu Gly Ser Tyr Leu Gly Ile Tyr Gln Gly Ala
 885 890 895
 Val Glu Lys Asn Phe Thr Pro Tyr Leu Arg Pro Gln Glu Val Gly Asn
 900 905 910
 Arg Ser Lys Val Arg Tyr Tyr Gln Leu Phe Asp Glu Glu Gly Gly Leu
 915 920 925
 Glu Phe Thr Ala Asn Gly Ala Asn Leu Asn Leu Ser Ala Leu Pro Tyr
 930 935 940
 Ser Ala Ala Gln Ile Glu Ala Ala Asp His Ala Phe Glu Leu Thr Asn
 945 950 955 960
 Asn Tyr Thr Trp Val Arg Ala Leu Ala Ala Gln Met Gly Val Gly Gly
 965 970 975
 Asp Asp Ser Trp Gly Gln Lys Val His Pro Glu Phe Cys Leu Asp Ala

980	985	990
Gln Glu Ala Arg Gln Leu Lys	Leu Val Ile Gln Pro	Leu Phe Thr Glu
995	1000	1005
<210> 32		
<211> 1049		
<212> PRT		
<213> 青春双歧杆菌		
<400> 32		
Met Ala Asp Thr Ala Glu Leu Ala Ile Val His Ala Thr Thr Ala Ser		
1	5	10
Ala Ser Trp Leu Thr Asp Pro Thr Val Phe Ala Ala Asn Arg Lys Pro		
20	25	30
Ala His Ser Ser His Arg Tyr Val Ile Gly Glu Thr Ser Glu Pro Lys		
35	40	45
Gln Ser Leu Asp Gly Glu Trp Lys Val Arg Ile Glu Gln Ala Arg Asn		
50	55	60
Val Asp Val Glu Ser Ala Pro Phe Ala Ala Val Asp Phe Glu Asp Gly		
65	70	75
Asp Phe Gly Ala Ile Glu Val Pro Gly His Leu Gln Met Ala Gly Tyr		
85	90	95
Leu Lys Asn Lys Tyr Val Asn Ile Gln Tyr Pro Trp Asp Gly His Glu		
100	105	110
Asp Pro Gln Ala Pro Asn Ile Pro Glu Asn Asn His Val Ala Ile Tyr		
115	120	125
Arg Arg Arg Phe Ala Leu Asp Ala Gln Leu Ala Arg Thr Leu Glu Asn		
130	135	140
Asp Gly Thr Val Ser Leu Thr Phe His Gly Ala Ala Thr Ala Ile Tyr		
145	150	155
Val Trp Leu Asp Gly Thr Phe Val Gly Tyr Gly Glu Asp Gly Phe Thr		
165	170	175
Pro Ser Glu Phe Asp Val Thr Glu Ala Leu Arg Asn Gly Asn Gly Asn		
180	185	190
Ala Ala Asp Ser Pro Glu Ala Glu His Thr Leu Thr Val Ala Cys Tyr		
195	200	205
Glu Tyr Ser Ser Ala Ser Trp Leu Glu Asp Gln Asp Phe Trp Arg Leu		
210	215	220
His Gly Leu Phe Arg Thr Val Glu Leu Ala Ala Gln Pro His Thr His		
225	230	235
Val Glu Thr Val Gln Leu Glu Ala Asp Tyr Thr Ala Ala Asp Thr Ala		

	245		250		255
Gly Thr Ala Asp Thr Ala Glu Leu Asn Ala Ala Leu Thr Leu Arg Asn					
	260		265		270
Pro Ala Asp Ala Met Thr Ile Glu Ser Thr Leu Arg Asp Gly Asp Gly					
	275		280		285
Asn Val Val Trp Glu Ser Thr Gln Ala Cys Asn Gly Glu Ile Ala Leu					
	290		295		300
Asn Ser Gly Lys Met Thr Asn Ile Ala Pro Trp Ser Ala Glu Ser Pro					
305		310		315	320
Thr Leu Tyr Thr Leu Thr Val Arg Val Val Gly His Asp Gly Ala Ile					
	325		330		335
Ile Glu Thr Val Thr Gln Lys Ile Gly Phe Arg Thr Phe Arg Ile Glu					
	340		345		350
Asn Gly Ile Met Thr Leu Asn Gly Lys Arg Ile Val Phe Lys Gly Ala					
	355		360		365
Asp Arg His Glu Phe Asp Ala Lys Arg Gly Arg Ala Ile Thr Arg Glu					
	370		375		380
Asp Met Leu Ser Asp Val Val Phe Cys Lys Arg His Asn Ile Asn Ala					
385		390		395	400
Ile Arg Thr Ser His Tyr Pro Asn Gln Glu Tyr Trp Tyr Asp Leu Cys					
	405		410		415
Asp Glu Tyr Gly Leu Tyr Leu Ile Asp Glu Thr Asn Met Glu Thr His					
	420		425		430
Gly Thr Trp Val Ala Asn Asn Val Glu Arg Pro Glu Asp Gly Ile Pro					
	435		440		445
Gly Ser Arg Pro Glu Trp Glu Asp Ala Cys Val Asp Arg Ile Asn Ser					
	450		455		460
Met Met Arg Arg Asp Tyr Asn His Pro Ser Val Leu Ile Trp Ser Leu					
465		470		475	480
Gly Asn Glu Ser Ser Ala Gly Glu Val Phe Arg Ala Met Tyr Arg His					
	485		490		495
Ala His Thr Ile Asp Pro Asn Arg Pro Val His Tyr Glu Gly Ser Val					
	500		505		510
His Met Arg Glu Phe Glu Asp Val Thr Asp Ile Glu Ser Arg Met Tyr					
	515		520		525
Ala His Ala Asp Glu Ile Glu Arg Tyr Leu Asn Asp Gly Ser Pro Ala					
	530		535		540
His Thr Asp Gly Pro Lys Lys Pro Tyr Ile Ser Cys Glu Tyr Met His					
545		550		555	560

Ala Met Gly Asn Ser Cys Gly Asn Met Asp Glu Tyr Thr Ala Leu Glu
 565 570 575
 Arg Tyr Pro Met Tyr Gln Gly Gly Phe Ile Trp Asp Phe Ile Asp Gln
 580 585 590
 Ala Ile Glu Thr Lys Leu Pro Asp Gly Thr Thr Arg Met Cys Tyr Gly
 595 600 605
 Gly Asp Phe Gly Asp Arg Pro Ser Asp Tyr Glu Phe Ser Gly Asp Gly
 610 615 620
 Leu Leu Phe Ala Asp Arg Thr Pro Ser Pro Lys Ala Gln Glu Val Lys
 625 630 635 640
 Gln Leu Tyr Ala Asn Val Lys Ile Ala Val Ser Val Asp Glu Ala Arg
 645 650 655
 Ile Thr Asn Asp Asn Leu Phe Val Ser Thr Gly Asp Tyr Arg Phe Val
 660 665 670
 Leu Arg Ile Leu Ala Asp Gly Lys Pro Val Trp Ser Thr Thr Arg Arg
 675 680 685
 Phe Asp Val Ala Ala Gly Glu Ser Ala Ser Phe Glu Val Asp Trp Pro
 690 695 700
 Val Asp Asp Tyr Arg Ser Asn Ala Glu Glu Leu Val Leu Glu Val Ser
 705 710 715 720
 Gln Gln Leu Gly Asn Ala Cys Asp Trp Ala Pro Ala Gly Tyr Glu Leu
 725 730 735
 Ala Phe Gly Gln Cys Val Val Ala Gly Ala Lys Thr Thr Ala Asp Ala
 740 745 750
 Val Asp Ala Ala Gly Ala Pro Ala Asp Gly Thr Val Thr Leu Gly Arg
 755 760 765
 Trp Asn Ala Gly Val Arg Gly Gln Gly Arg Glu Ala Leu Phe Ser Arg
 770 775 780
 Thr Gln Gly Gly Met Val Ser Tyr Thr Phe Gly Glu Arg Glu Phe Val
 785 790 795 800
 Leu Arg Arg Pro Ser Ile Thr Thr Phe Arg Pro Leu Thr Asp Asn Asp
 805 810 815
 Arg Gly Ala Gly His Ala Phe Glu Arg Ala Ala Trp Ala Val Ala Gly
 820 825 830
 Lys Tyr Ala Arg Cys Val Asp Cys Ala Ile Ala Asn Arg Gly Glu Asn
 835 840 845
 Ala Val Glu Ala Thr Tyr Thr Tyr Glu Leu Ala Ile Pro Gln Arg Thr
 850 855 860
 Lys Val Thr Val Arg Tyr Val Ala Asp Thr Ala Gly Leu Val Ser Leu

865		870		875		880									
Asp	Val	Glu	Tyr	Pro	Gly	Glu	Lys	Asn	Gly	Asp	Leu	Pro	Thr	Ile	Pro
		885		890		895									
Ala	Phe	Gly	Ile	Glu	Trp	Ala	Leu	Pro	Val	Glu	Tyr	Ala	Asn	Leu	Arg
		900		905		910									
Phe	Tyr	Gly	Ala	Gly	Pro	Glu	Glu	Thr	Tyr	Ala	Asp	Arg	Arg	His	Ala
		915		920		925									
Lys	Leu	Gly	Val	Trp	Ser	Thr	Thr	Ala	Gly	Asp	Asp	Cys	Ala	Pro	Tyr
		930		935		940									
Leu	Leu	Pro	Gln	Glu	Thr	Gly	Asn	His	Glu	Asp	Val	Arg	Trp	Ala	Glu
945		950		955		960									
Ile	Thr	Asp	Asp	Ser	Gly	His	Gly	Val	Arg	Val	Lys	Arg	Gly	Ala	Gly
		965		970		975									
Ala	Lys	Pro	Phe	Ala	Met	Ser	Leu	Leu	Pro	Tyr	Ser	Ser	Thr	Met	Leu
		980		985		990									
Glu	Glu	Ala	Leu	His	Gln	Asp	Glu	Leu	Pro	Lys	Pro	Arg	His	Met	Phe
		995		1000		1005									
Leu	Arg	Leu	Leu	Ala	Ala	Gln	Met	Gly	Val	Gly	Gly	Asp	Asp	Ser	
1010		1015		1020											
Trp	Met	Ser	Pro	Val	His	Glu	Gln	Tyr	Gln	Leu	Pro	Ala	Asp	Gln	
1025		1030		1035											
Pro	Leu	Ser	Leu	Asn	Val	Gln	Leu	Lys	Leu	Phe					
1040		1045													
<210>	33														
<211>	1023														
<212>	PRT														
<213>	青春双歧杆菌														
<400>	33														
Met	Ala	Asn	Glu	Thr	Arg	Ile	Glu	His	Ala	Ser	Glu	Thr	Trp	Leu	Ala
1		5		10		15									
Asp	Ser	Thr	Val	Phe	Glu	Val	Asn	Arg	Val	Pro	Ala	His	Ser	Asp	His
		20		25		30									
Lys	Cys	Tyr	Ala	His	Asp	Ser	Gln	Thr	Asn	Glu	Trp	Ser	Asp	Leu	Arg
		35		40		45									
Gln	Ser	Leu	Asp	Gly	Glu	Trp	Arg	Val	Glu	Val	Val	Gln	Ala	Ser	Asp
50		55		60											
Ile	Glu	Phe	Asn	Glu	Glu	Pro	Phe	Val	Arg	Glu	Asn	Phe	Asp	Asp	Ser
65		70		75		80									
Ala	Phe	Glu	Arg	Ile	Gln	Val	Pro	Gly	His	Leu	Gln	Met	Ala	Gly	Leu

	85	90	95
Met Asn Asn Lys Tyr Val Asn Ile Gln Tyr Pro Trp Asp Gly His Glu			
	100	105	110
Asn Pro Ala Glu Pro Asn Ile Pro Glu Asn Asn His Val Ala Leu Tyr			
	115	120	125
Arg Lys Thr Phe Thr Met Ala Asn Arg Leu Ala Asp Thr Lys Asn Ala			
	130	135	140
Gly Gly Thr Val Ser Ile Val Phe His Gly Met Ala Thr Ala Ile Tyr			
145	150	155	160
Val Trp Val Asn Gly Met Phe Val Gly Tyr Gly Glu Asp Gly Phe Thr			
	165	170	175
Pro Asn Glu Phe Asp Ile Thr Glu Met Leu His Asp Gly Glu Asn Val			
	180	185	190
Val Ala Val Ala Cys Tyr Glu Tyr Ser Ser Ala Ser Trp Leu Glu Asp			
	195	200	205
Gln Asp Phe Trp Arg Leu His Gly Leu Phe Arg Ser Val Glu Leu Ala			
	210	215	220
Ala Gln Pro His Val His Ile Glu Asn Met Gln Ile Glu Ser Asp Trp			
225	230	235	240
Asp Pro Glu Ser Gly Ser Ala Ser Leu Asp Ala Ala Leu Thr Val Arg			
	245	250	255
Asn Ala Ala Asp Ala Ala Thr Ile Ser Ala Thr Leu Lys Asp Ser Asp			
	260	265	270
Gly Asn Val Val Trp Glu Thr Ala Asn Cys Ala Asp Pro Asp Thr Ser			
	275	280	285
Ile Ser Thr Gly Ser Leu Asn Gly Ile Arg Pro Trp Ser Ala Glu Asp			
	290	295	300
Pro Val Leu Tyr Glu Phe Glu Val Thr Val Ile Asp His Ala Gly Asn			
305	310	315	320
Ile Ala Glu Val Ala Val Gln Lys Val Gly Phe Arg Arg Phe Arg Ile			
	325	330	335
Glu Asp Gly Ile Met Thr Ile Asn Gly Lys Arg Ile Val Phe Lys Gly			
	340	345	350
Ala Asp Arg His Glu Phe Asp Pro Lys Arg Gly Arg Ala Ile Thr Glu			
	355	360	365
Gln Asp Met Ile Asp Asp Val Val Phe Cys Lys Arg His Asn Leu Asn			
	370	375	380
Ala Ile Arg Thr Ser His Tyr Pro Asn Gln Glu Arg Trp Tyr Glu Leu			
385	390	395	400

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

705	710	715	720
Val Val Ala Gly Ser Lys Lys Ala Ser Ala Pro Val Lys Pro Val Asp			
	725	730	735
Gly Ile Val Thr Val Gly Arg Trp Asn Val Gly Val Gln Gly Ser Gly			
	740	745	750
Arg Glu Val Leu Leu Ser Arg Thr Gln Gly Gly Leu Val Ser Tyr Thr			
	755	760	765
Phe Asn Asn Arg Glu Phe Val Leu Arg Arg Pro Ala Val Thr Thr Phe			
	770	775	780
Arg Ala Leu Thr Asp Asn Asp Arg Gly Ala Gly His Gly Phe Glu Arg			
785	790	795	800
Ala Gln Trp Leu Gly Ala Gly Arg Tyr Ala Arg Cys Ile Gly Asn Glu			
	805	810	815
Ile Glu Gln Ile Asp Glu Asn Thr Val Lys Ala Ser Tyr Thr Tyr Glu			
	820	825	830
Leu Ala Thr Pro Gln Arg Thr Lys Val Thr Val Ser Tyr Thr Ala Asp			
	835	840	845
Thr Thr Gly Arg Val Asn Leu His Val Glu Tyr Pro Gly Glu Pro Gly			
	850	855	860
Asp Leu Pro Thr Ile Pro Ala Phe Gly Ile Glu Trp Thr Leu Pro Val			
865	870	875	880
Gln Tyr Ser Asn Leu Arg Phe Phe Gly Ala Gly Pro Glu Glu Thr Tyr			
	885	890	895
Gln Asp Arg Lys His Ala Lys Leu Gly Val Trp Ser Thr Asp Ala Phe			
	900	905	910
Lys Asp His Ala Pro Tyr Leu Met Pro Gln Glu Thr Gly Asn His Glu			
	915	920	925
Asp Val Arg Trp Ala Glu Ile Thr Asp Glu Lys Gly His Gly Leu Arg			
	930	935	940
Ile Ser Arg Ala Glu Gly Ala Glu Pro Phe Ala Met Ser Leu Gln Pro			
945	950	955	960
Tyr Ser Ser Phe Met Leu Glu Glu Ala Gln His Gln Asp Glu Leu Pro			
	965	970	975
Ala Pro Lys His Met Phe Leu Arg Val Leu Ala Glu Gln Met Gly Val			
	980	985	990
Gly Gly Asp Asp Ser Trp Met Ser Pro Val His Pro Gln Tyr His Ile			
	995	1000	1005
Pro Ala Asp Gln Pro Ile Ser Leu Asp Val Asp Leu Asp Leu Ile			
	1010	1015	1020

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

Ser Ile Lys Asn Pro Asn Leu Tyr Thr Val Arg Thr Glu Val Leu Asn
 275 280 285
 Gly Asp Thr Val Leu Asp Thr Tyr Asp Thr Glu Tyr Gly Phe Arg Trp
 290 295 300
 Thr Gly Phe Asp Ala Thr Ser Gly Phe Ser Leu Asn Gly Glu Lys Val
 305 310 315 320
 Lys Leu Lys Gly Val Ser Met His His Asp Gln Gly Ser Leu Gly Ala
 325 330 335
 Val Ala Asn Arg Arg Ala Ile Glu Arg Gln Val Glu Ile Leu Gln Lys
 340 345 350
 Met Gly Val Asn Ser Ile Arg Thr Thr His Asn Pro Ala Ala Lys Ala
 355 360 365
 Leu Ile Asp Val Cys Asn Glu Lys Gly Val Leu Val Val Glu Glu Val
 370 375 380
 Phe Asp Met Trp Asn Arg Ser Lys Asn Gly Asn Thr Glu Asp Tyr Gly
 385 390 395 400
 Lys Trp Phe Gly Gln Thr Ile Ala Gly Asp Asn Ala Val Leu Gly Gly
 405 410 415
 Asp Lys Asp Glu Thr Trp Ala Lys Phe Asp Leu Thr Ser Thr Ile Asn
 420 425 430
 Arg Asp Arg Asn Ala Pro Ser Val Ile Met Trp Ser Leu Gly Asn Glu
 435 440 445
 Met Met Glu Gly Ile Ser Gly Ser Val Ser Asp Phe Pro Ala Thr Ser
 450 455 460
 Ala Lys Leu Val Ala Trp Thr Lys Ala Ala Asp Ser Thr Arg Pro Met
 465 470 475 480
 Thr Tyr Gly Asp Asn Lys Ile Lys Ala Asn Trp Asn Glu Ser Asn Thr
 485 490 495
 Met Gly Asp Asn Leu Thr Ala Asn Gly Gly Val Val Gly Thr Asn Tyr
 500 505 510
 Ser Asp Gly Ala Asn Tyr Asp Lys Ile Arg Thr Thr His Pro Ser Trp
 515 520 525
 Ala Ile Tyr Gly Ser Glu Thr Ala Ser Ala Ile Asn Ser Arg Gly Ile
 530 535 540
 Tyr Asn Arg Thr Thr Gly Gly Ala Gln Ser Ser Asp Lys Gln Leu Thr
 545 550 555 560
 Ser Tyr Asp Asn Ser Ala Val Gly Trp Gly Ala Val Ala Ser Ser Ala
 565 570 575
 Trp Tyr Asp Val Val Gln Arg Asp Phe Val Ala Gly Thr Tyr Val Trp

	580		585		590
Thr Gly Phe Asp Tyr Leu Gly Glu Pro Thr Pro Trp Asn Gly Thr Gly					
	595		600		605
Ser Gly Ala Val Gly Ser Trp Pro Ser Pro Lys Asn Ser Tyr Phe Gly					
	610		615		620
Ile Val Asp Thr Ala Gly Phe Pro Lys Asp Thr Tyr Tyr Phe Tyr Gln					
625		630		635	640
Ser Gln Trp Asn Asp Asp Val His Thr Leu His Ile Leu Pro Ala Trp					
	645		650		655
Asn Glu Asn Val Val Ala Lys Gly Ser Gly Asn Lys Val Pro Val Val					
	660		665		670
Val Tyr Thr Asp Ala Ala Lys Val Lys Leu Tyr Phe Thr Pro Lys Gly					
	675		680		685
Ser Thr Glu Lys Arg Leu Ile Gly Glu Lys Ser Phe Thr Lys Lys Thr					
	690		695		700
Thr Ala Ala Gly Tyr Thr Tyr Gln Val Tyr Glu Gly Thr Asp Lys Asp					
705		710		715	720
Ser Thr Ala His Lys Asn Met Tyr Leu Thr Trp Asn Val Pro Trp Ala					
	725		730		735
Glu Gly Thr Ile Ser Ala Glu Ala Tyr Asp Glu Asn Asn Arg Leu Ile					
	740		745		750
Pro Glu Gly Ser Thr Glu Gly Asn Ala Ser Val Thr Thr Thr Gly Lys					
	755		760		765
Ala Ala Lys Leu Lys Ala Asp Ala Asp Arg Lys Thr Ile Thr Ala Asp					
	770		775		780
Gly Lys Asp Leu Ser Tyr Ile Glu Val Asp Val Thr Asp Ala Asn Gly					
785		790		795	800
His Ile Val Pro Asp Ala Ala Asn Arg Val Thr Phe Asp Val Lys Gly					
	805		810		815
Ala Gly Lys Leu Val Gly Val Asp Asn Gly Ser Ser Pro Asp His Asp					
	820		825		830
Ser Tyr Gln Ala Asp Asn Arg Lys Ala Phe Ser Gly Lys Val Leu Ala					
	835		840		845
Ile Val Gln Ser Thr Lys Glu Ala Gly Glu Ile Thr Val Thr Ala Lys					
	850		855		860
Ala Asp Gly Leu Gln Ser Ser Thr Val Lys Ile Ala Thr Thr Ala Val					
865		870		875	880
Pro Gly Thr Ser Thr Glu Lys Thr Val Arg Ser Phe Tyr Tyr Ser Arg					
	885		890		895

Asn Tyr Tyr Val Lys Thr Gly Asn Lys Pro Ile Leu Pro Ser Asp Val
 900 905 910
 Glu Val Arg Tyr Ser Asp Gly Thr Ser Asp Arg Gln Asn Val Thr Trp
 915 920 925
 Asp Ala Val Ser Asp Asp Gln Ile Ala Lys Ala Gly Ser Phe Ser Val
 930 935 940
 Ala Gly Thr Val Ala Gly Gln Lys Ile Ser Val Arg Val Thr Met Ile
 945 950 955 960
 Asp Glu Ile Gly Ala Leu Leu Asn Tyr Ser Ala Ser Thr Pro Val Gly
 965 970 975
 Thr Pro Ala Val Leu Pro Gly Ser Arg Pro Ala Val Leu Pro Asp Gly
 980 985 990
 Thr Val Thr Ser Ala Asn Phe Ala Val His Trp Thr Lys Pro Ala Asp
 995 1000 1005
 Thr Val Tyr Asn Thr Ala Gly Thr Val Lys Val Pro Gly Thr Ala
 1010 1015 1020
 Thr Val Phe Gly Lys Glu Phe Lys Val Thr Ala Thr Ile Arg Val
 1025 1030 1035
 Gln Arg Ser Gln Val Thr Ile Gly Ser Ser Val Ser Gly Asn Ala
 1040 1045 1050
 Leu Arg Leu Thr Gln Asn Ile Pro Ala Asp Lys Gln Ser Asp Thr
 1055 1060 1065
 Leu Asp Ala Ile Lys Asp Gly Ser Thr Thr Val Asp Ala Asn Thr
 1070 1075 1080
 Gly Gly Gly Ala Asn Pro Ser Ala Trp Thr Asn Trp Ala Tyr Ser
 1085 1090 1095
 Lys Ala Gly His Asn Thr Ala Glu Ile Thr Phe Glu Tyr Ala Thr
 1100 1105 1110
 Glu Gln Gln Leu Gly Gln Ile Val Met Tyr Phe Phe Arg Asp Ser
 1115 1120 1125
 Asn Ala Val Arg Phe Pro Asp Ala Gly Lys Thr Lys Ile Gln Ile
 1130 1135 1140
 Ser Ala Asp Gly Lys Asn Trp Thr Asp Leu Ala Ala Thr Glu Thr
 1145 1150 1155
 Ile Ala Ala Gln Glu Ser Ser Asp Arg Val Lys Pro Tyr Thr Tyr
 1160 1165 1170
 Asp Phe Ala Pro Val Gly Ala Thr Phe Val Lys Val Thr Val Thr
 1175 1180 1185
 Asn Ala Asp Thr Thr Thr Pro Ser Gly Val Val Cys Ala Gly Leu

1190	1195	1200
Thr Glu Ile Glu Leu Lys	Thr Ala Thr Ser Lys	Phe Val Thr Asn
1205	1210	1215
Thr Ser Ala Ala Leu Ser	Ser Leu Thr Val Asn	Gly Thr Lys Val
1220	1225	1230
Ser Asp Ser Val Leu Ala	Ala Gly Ser Tyr Asn	Thr Pro Ala Ile
1235	1240	1245
Ile Ala Asp Val Lys Ala	Glu Gly Glu Gly Asn	Ala Ser Val Thr
1250	1255	1260
Val Leu Pro Ala His Asp	Asn Val Ile Arg Val	Ile Thr Glu Ser
1265	1270	1275
Glu Asp His Val Thr Arg	Lys Thr Phe Thr Ile	Asn Leu Gly Thr
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Glu Gln Glu Phe Pro Ala	Asp Ser Asp Glu Arg	Asp
1295	1300	1305
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Glu Ser Leu Asn Gly Pro	Trp Ala Phe Ala Leu	Phe Asp Ala Pro Leu
	35	40
Asp Ala Pro Asp Ala Lys	Asn Leu Asp Trp Glu	Thr Ala Lys Lys Trp
	50	55
Ser Thr Ile Ser Val Pro	Ser His Trp Glu Leu	Gln Glu Asp Trp Lys
65	70	75
Tyr Gly Lys Pro Ile Tyr	Thr Asn Val Gln Tyr	Pro Ile Pro Ile Asp
	85	90
Ile Pro Asn Pro Pro Thr	Val Asn Pro Thr Gly	Val Tyr Ala Arg Thr
	100	105
Phe Glu Leu Asp Ser Lys	Ser Ile Glu Ser Phe	Glu His Arg Leu Arg
	115	120
Phe Glu Gly Val Asp Asn	Cys Tyr Glu Leu Tyr	Val Asn Gly Gln Tyr
	130	135
Val Gly Phe Asn Lys Gly	Ser Arg Asn Gly Ala	Glu Phe Asp Ile Gln

145	150	155	160
Lys Tyr Val Ser Glu Gly Glu Asn Leu Val Val Val Lys Val Phe Lys			
	165	170	175
Trp Ser Asp Ser Thr Tyr Ile Glu Asp Gln Asp Gln Trp Trp Leu Ser			
	180	185	190
Gly Ile Tyr Arg Asp Val Ser Leu Leu Lys Leu Pro Lys Lys Ala His			
	195	200	205
Ile Glu Asp Val Arg Val Thr Thr Thr Phe Val Asp Ser Gln Tyr Gln			
	210	215	220
Asp Ala Glu Leu Ser Val Lys Val Asp Val Gln Gly Ser Ser Tyr Asp			
225	230	235	240
His Ile Asn Phe Thr Leu Tyr Glu Pro Glu Asp Gly Ser Lys Val Tyr			
	245	250	255
Asp Ala Ser Ser Leu Leu Asn Glu Glu Asn Gly Asn Thr Thr Phe Ser			
	260	265	270
Thr Lys Glu Phe Ile Ser Phe Ser Thr Lys Lys Asn Glu Glu Thr Ala			
	275	280	285
Phe Lys Ile Asn Val Lys Ala Pro Glu His Trp Thr Ala Glu Asn Pro			
	290	295	300
Thr Leu Tyr Lys Tyr Gln Leu Asp Leu Ile Gly Ser Asp Gly Ser Val			
305	310	315	320
Ile Gln Ser Ile Lys His His Val Gly Phe Arg Gln Val Glu Leu Lys			
	325	330	335
Asp Gly Asn Ile Thr Val Asn Gly Lys Asp Ile Leu Phe Arg Gly Val			
	340	345	350
Asn Arg His Asp His His Pro Arg Phe Gly Arg Ala Val Pro Leu Asp			
	355	360	365
Phe Val Val Arg Asp Leu Ile Leu Met Lys Lys Phe Asn Ile Asn Ala			
	370	375	380
Val Arg Asn Ser His Tyr Pro Asn His Pro Lys Val Tyr Asp Leu Phe			
385	390	395	400
Asp Lys Leu Gly Phe Trp Val Ile Asp Glu Ala Asp Leu Glu Thr His			
	405	410	415
Gly Val Gln Glu Pro Phe Asn Arg His Thr Asn Leu Glu Ala Glu Tyr			
	420	425	430
Pro Asp Thr Lys Asn Lys Leu Tyr Asp Val Asn Ala His Tyr Leu Ser			
	435	440	445
Asp Asn Pro Glu Tyr Glu Val Ala Tyr Leu Asp Arg Ala Ser Gln Leu			
450	455	460	

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

770	775	780
Ser Ile Thr Phe Trp Arg Pro Pro Thr Asn Asn Asp Glu Pro Arg Asp		
785	790	795
Phe Lys Asn Trp Lys Lys Tyr Asn Ile Asp Leu Met Lys Gln Asn Ile		800
	805	810
His Gly Val Ser Val Glu Lys Gly Ser Asn Gly Ser Leu Ala Val Val		815
	820	825
Thr Val Asn Ser Arg Ile Ser Pro Val Val Phe Tyr Tyr Gly Phe Glu		830
	835	840
Thr Val Gln Lys Tyr Thr Ile Phe Ala Asn Lys Ile Asn Leu Asn Thr		845
	850	855
Ser Met Lys Leu Thr Gly Glu Tyr Gln Pro Pro Asp Phe Pro Arg Val		860
865	870	875
Gly Tyr Glu Phe Trp Leu Gly Asp Ser Tyr Glu Ser Phe Glu Trp Leu		880
	885	890
Gly Arg Gly Pro Gly Glu Ser Tyr Pro Asp Lys Lys Glu Ser Gln Arg		895
	900	905
Phe Gly Leu Tyr Asp Ser Lys Asp Val Glu Glu Phe Val Tyr Asp Tyr		910
	915	920
Pro Gln Glu Asn Gly Asn His Thr Asp Thr His Phe Leu Asn Ile Lys		925
	930	935
Phe Glu Gly Ala Gly Lys Leu Ser Ile Phe Gln Lys Glu Lys Pro Phe		940
945	950	955
Asn Phe Lys Ile Ser Asp Glu Tyr Gly Val Asp Glu Ala Ala His Ala		960
	965	970
Cys Asp Val Lys Arg Tyr Gly Arg His Tyr Leu Arg Leu Asp His Ala		975
	980	985
Ile His Gly Val Gly Ser Glu Ala Cys Gly Pro Ala Val Leu Asp Gln		990
	995	1000
Tyr Arg Leu Lys Ala Gln Asp Phe Asn Phe Glu Phe Asp Leu Ala		1005
	1010	1015
Phe Glu		1020
	1025	
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<223> n是第9位的脱氧尿苷残基
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cggcgtcaca ctttgctatg cc 22

<210> 41

<211> 20

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<213> 引物

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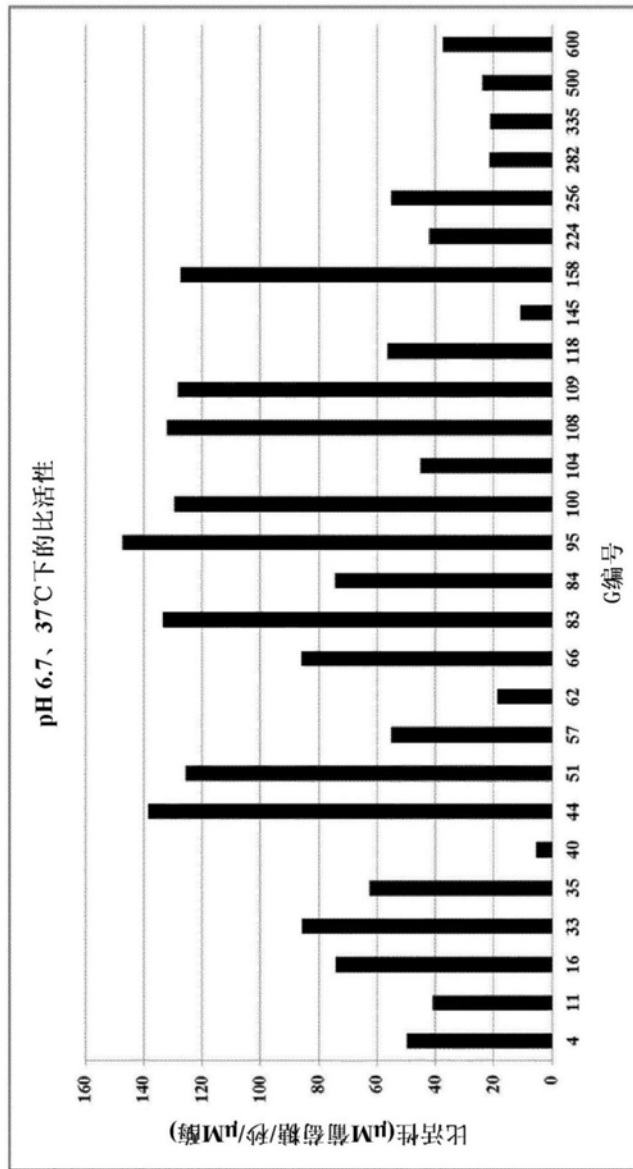


图1

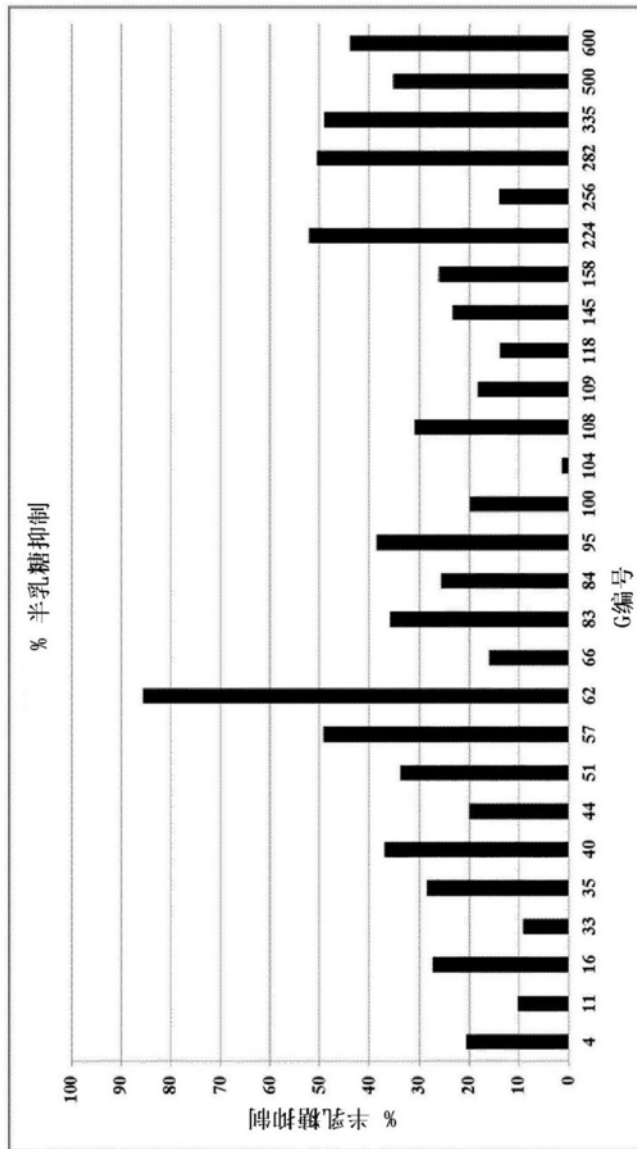


图2

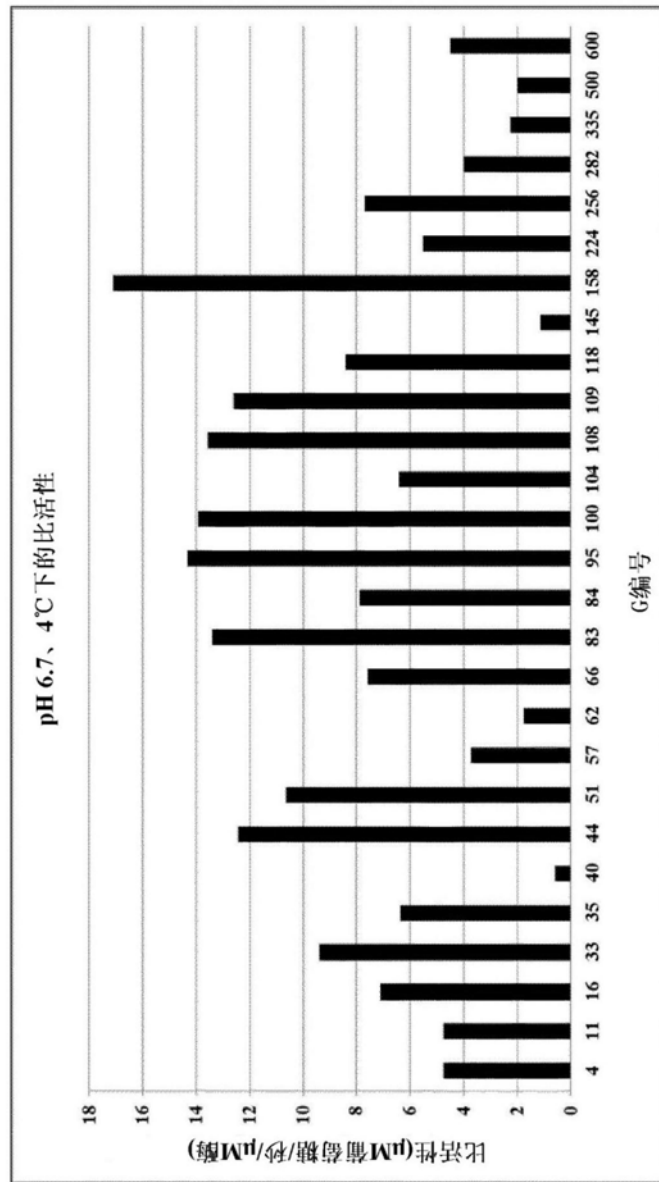


图3

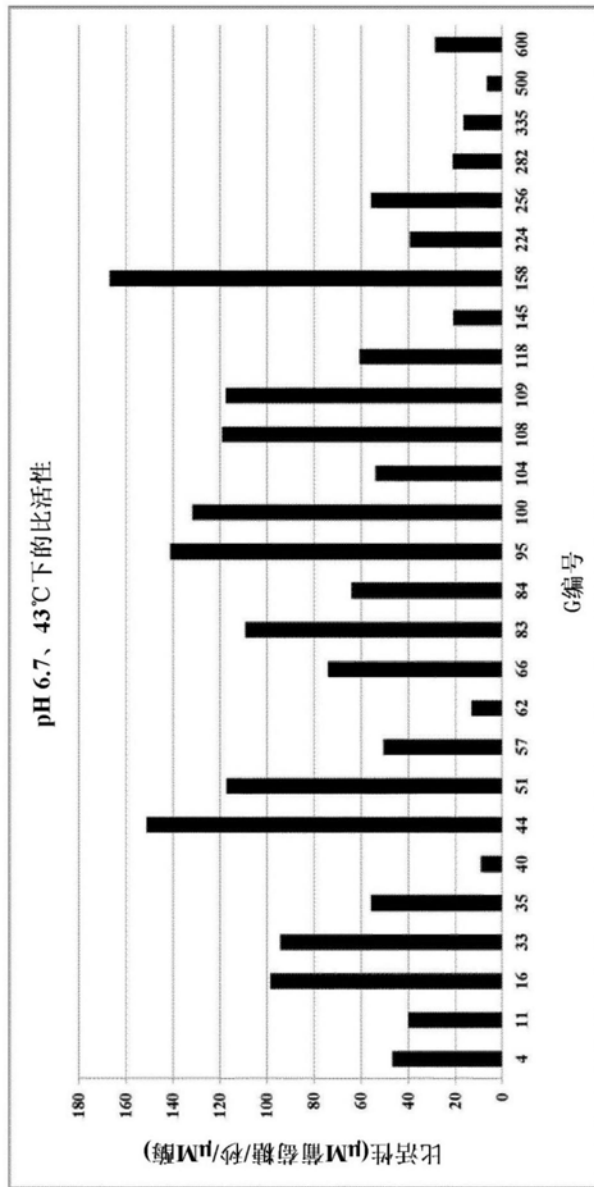


图4

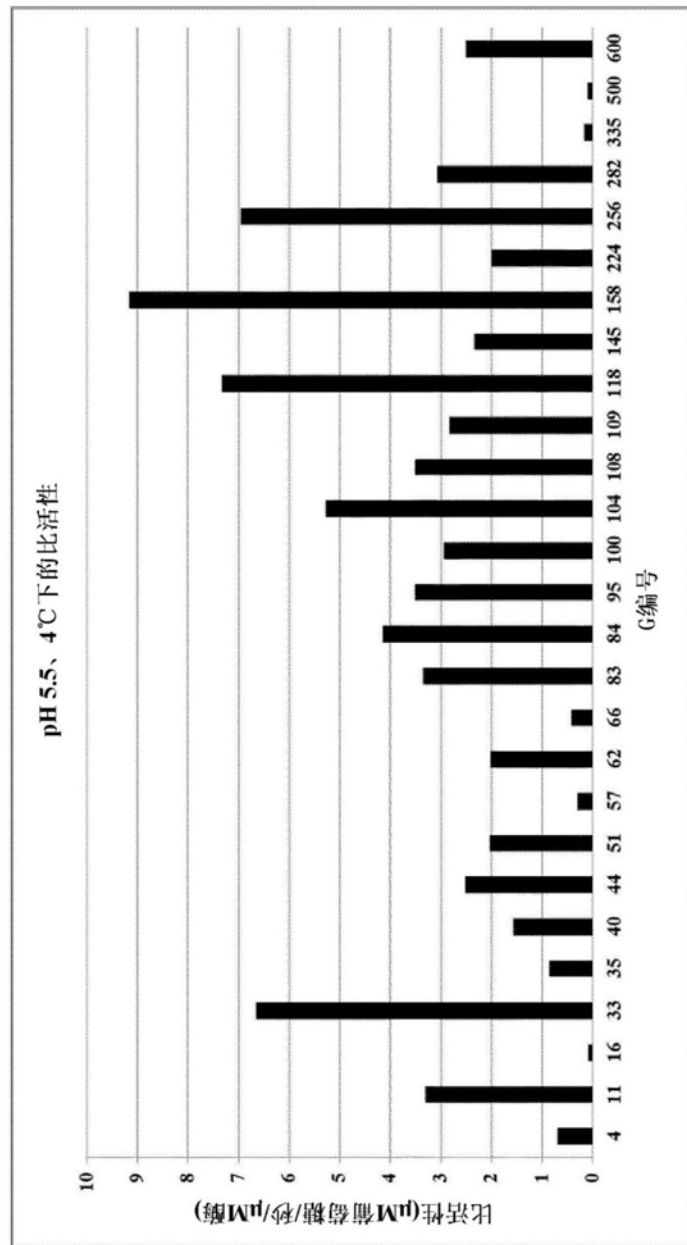


图5

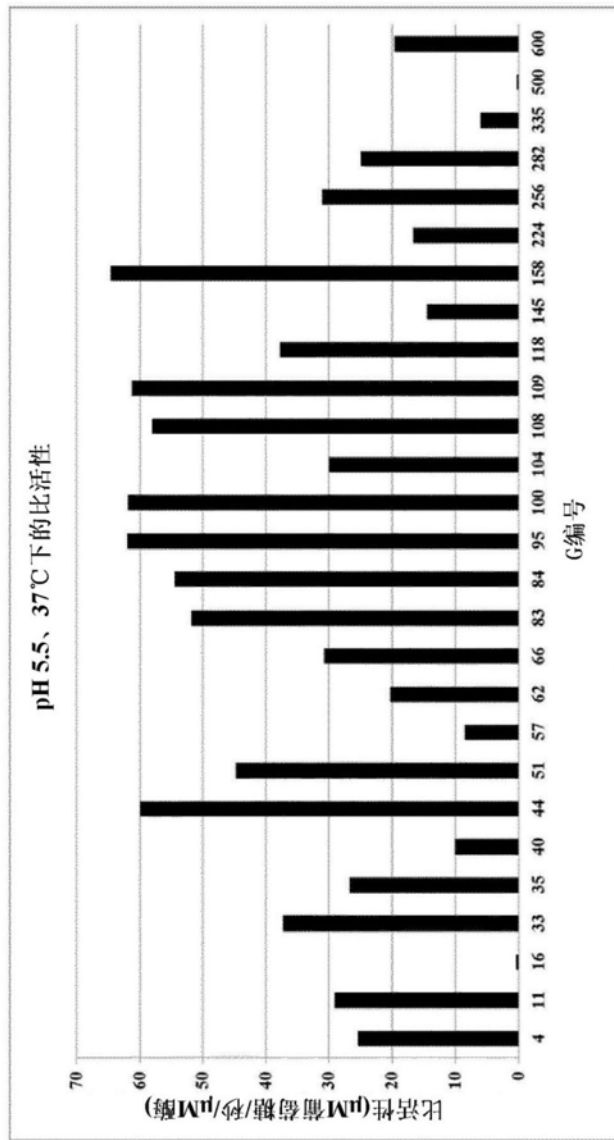


图6

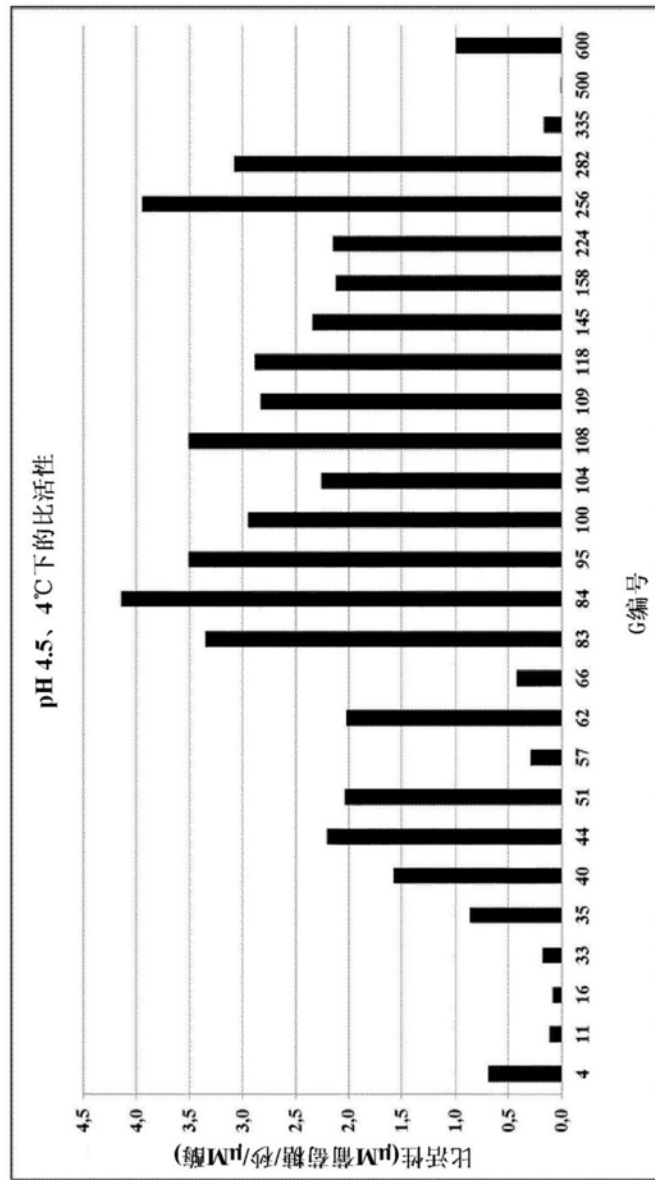


图8

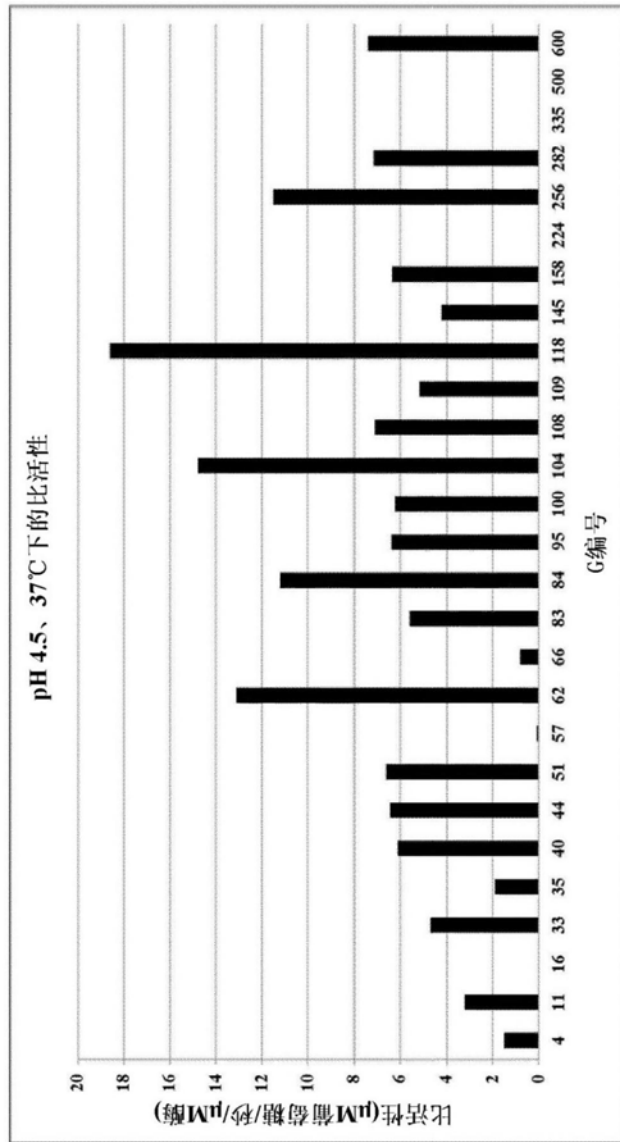


图9

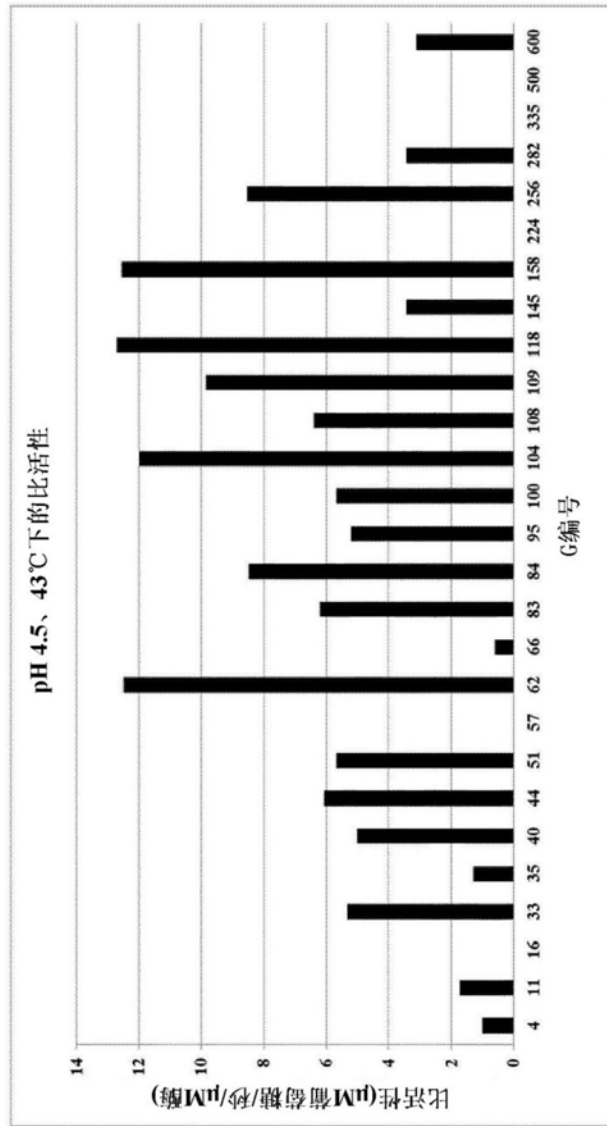


图10

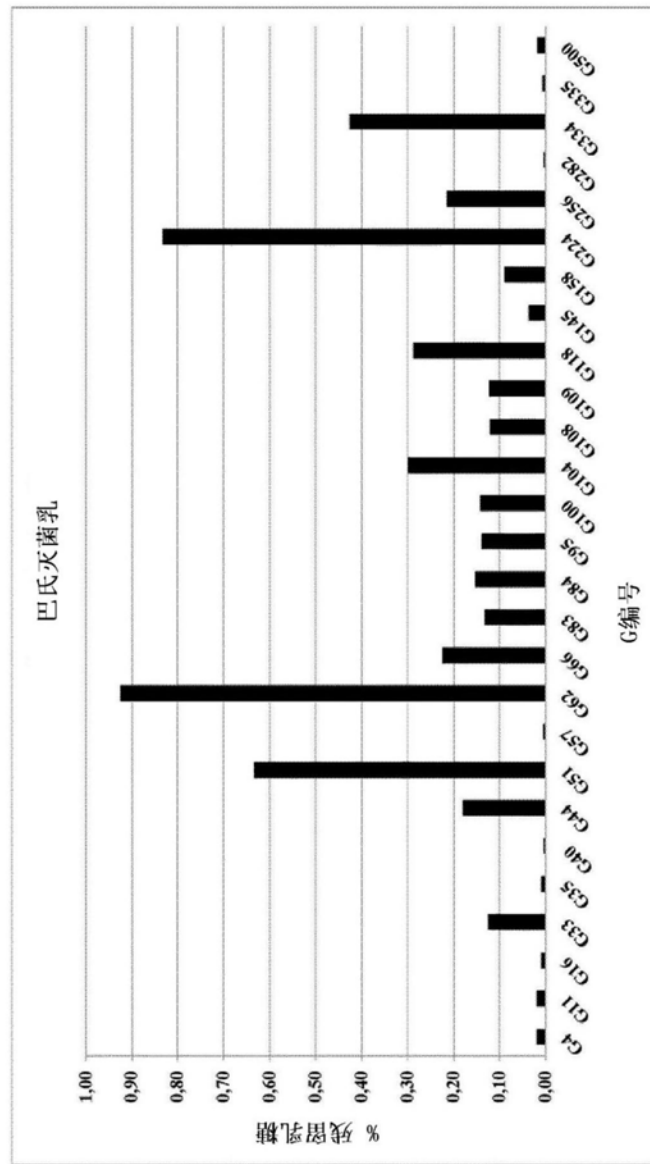


图11

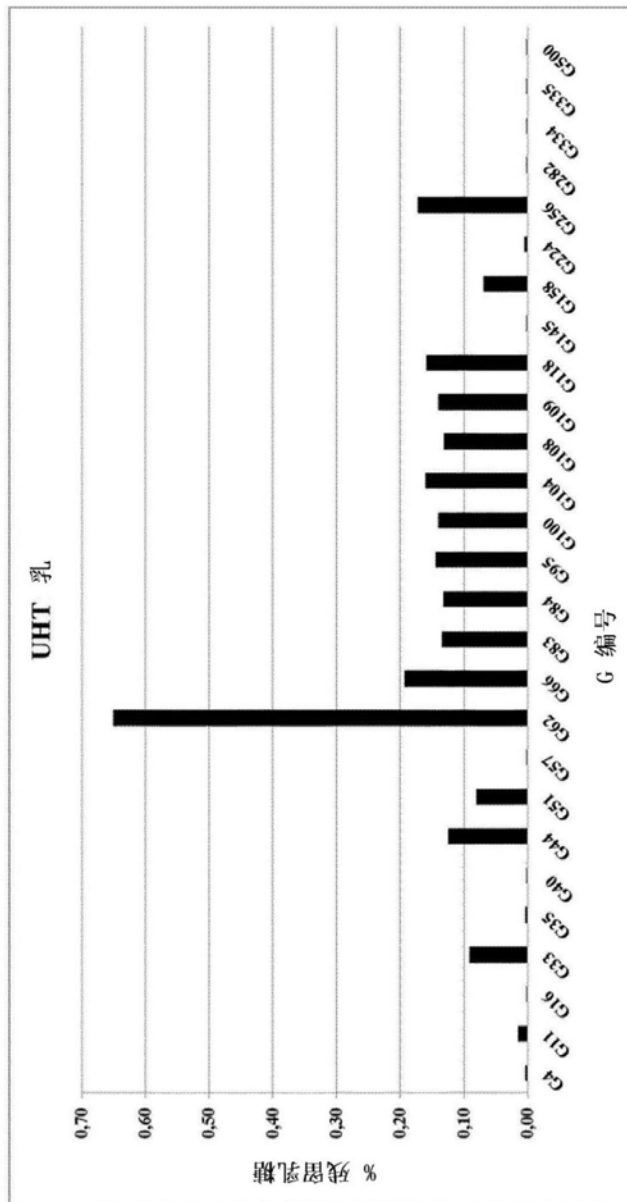


图12

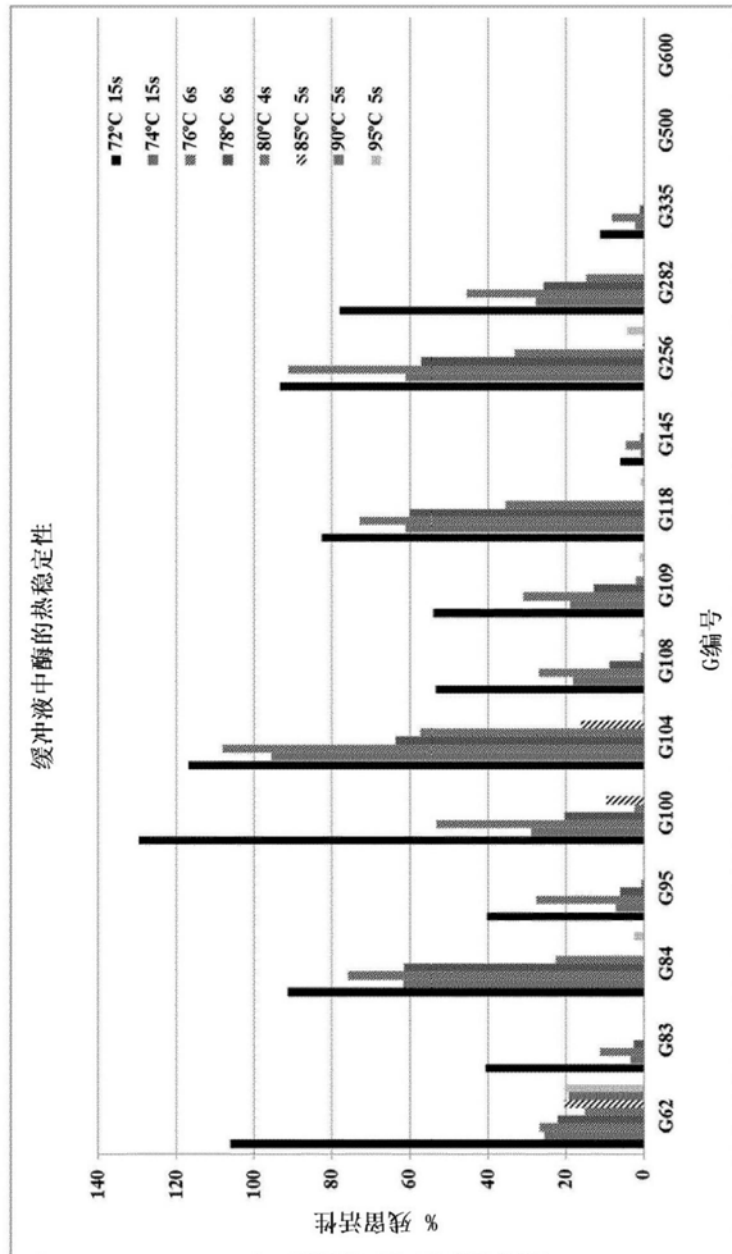


图13

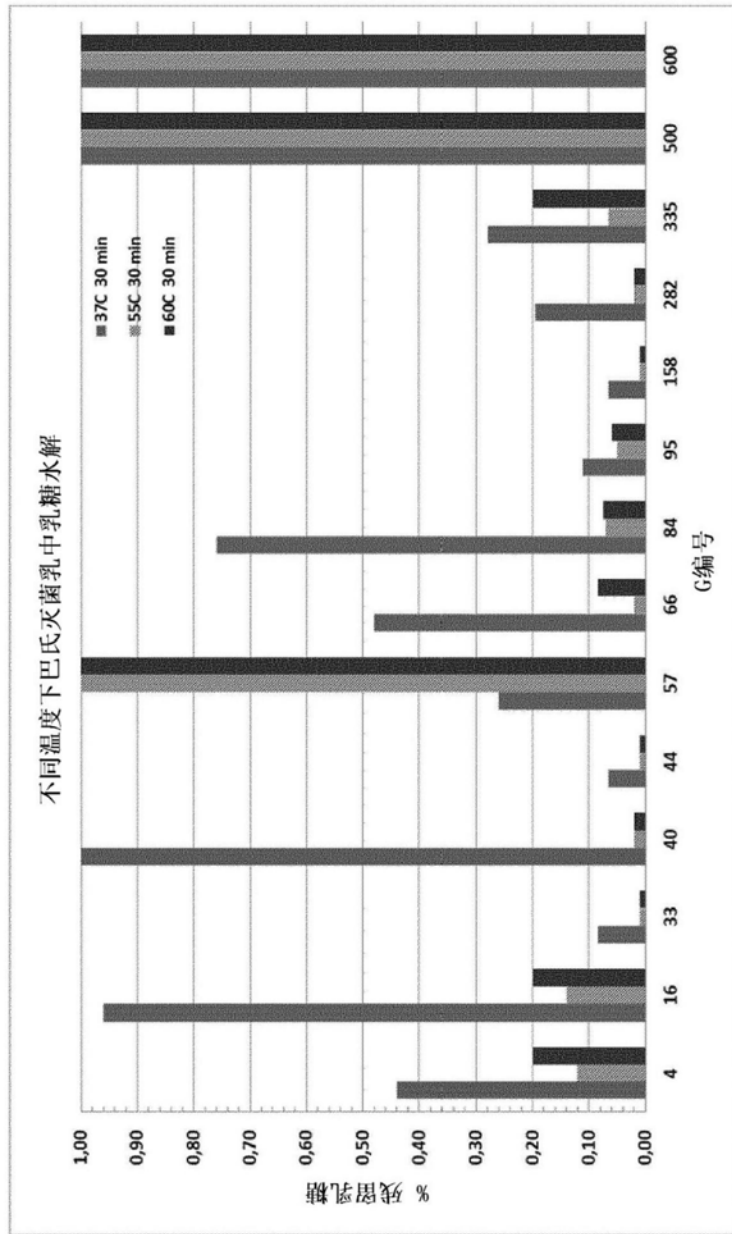


图14

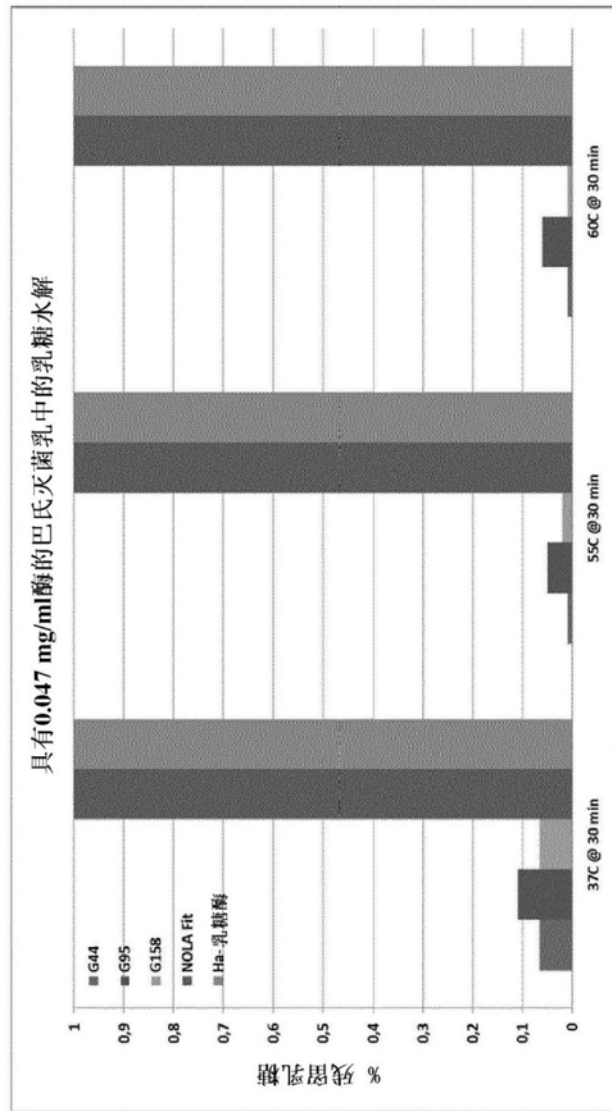


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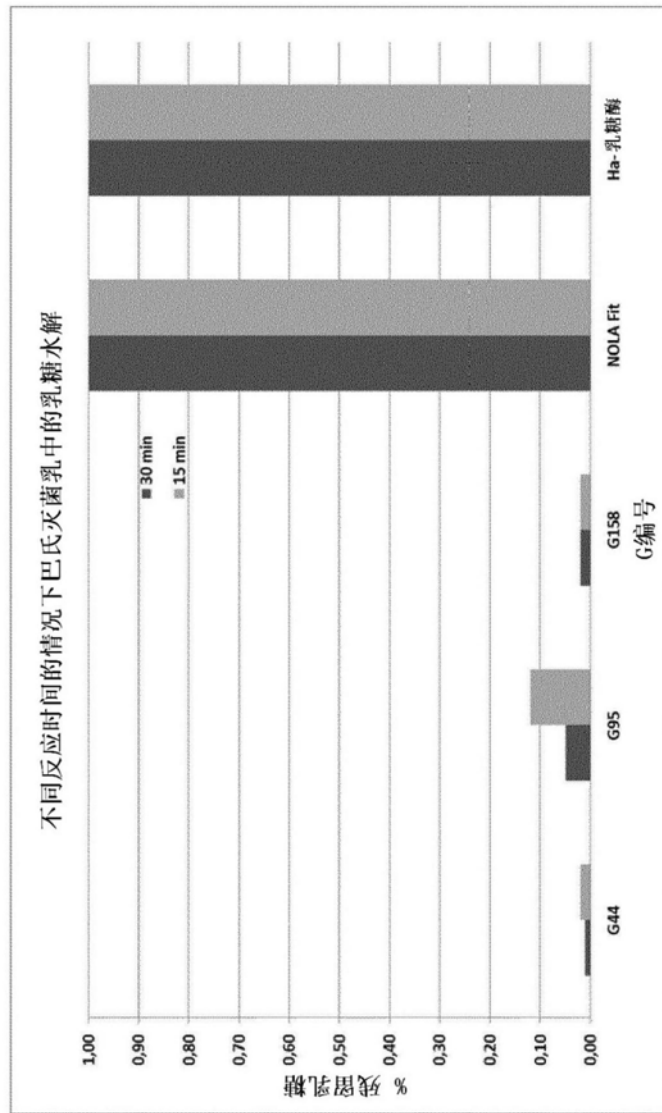


图16

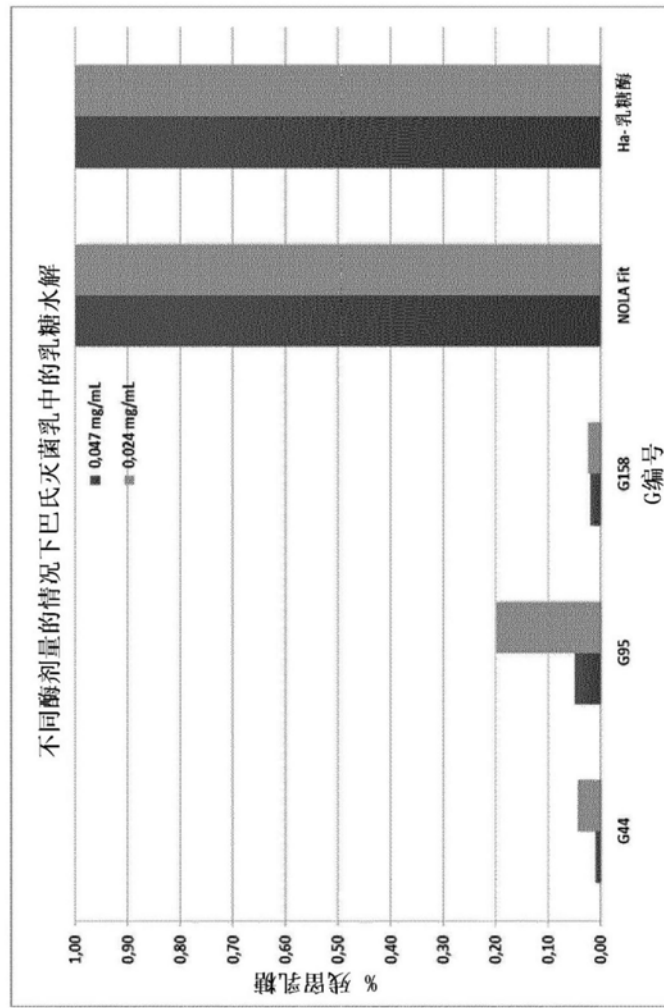


图17

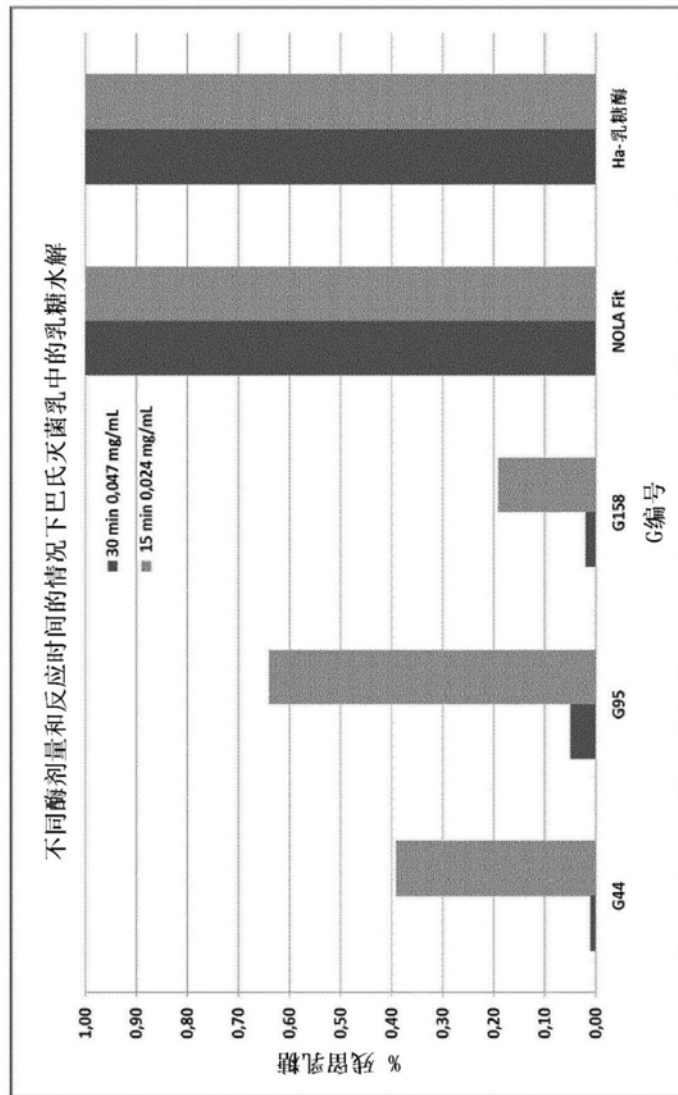


图18

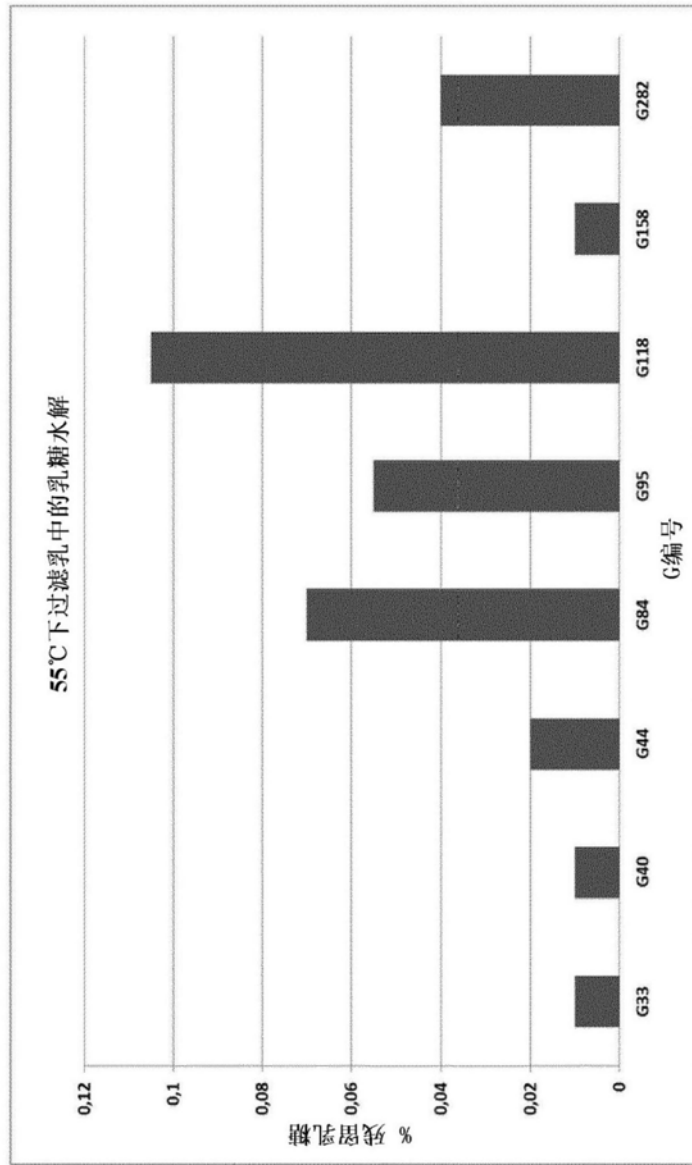


图19

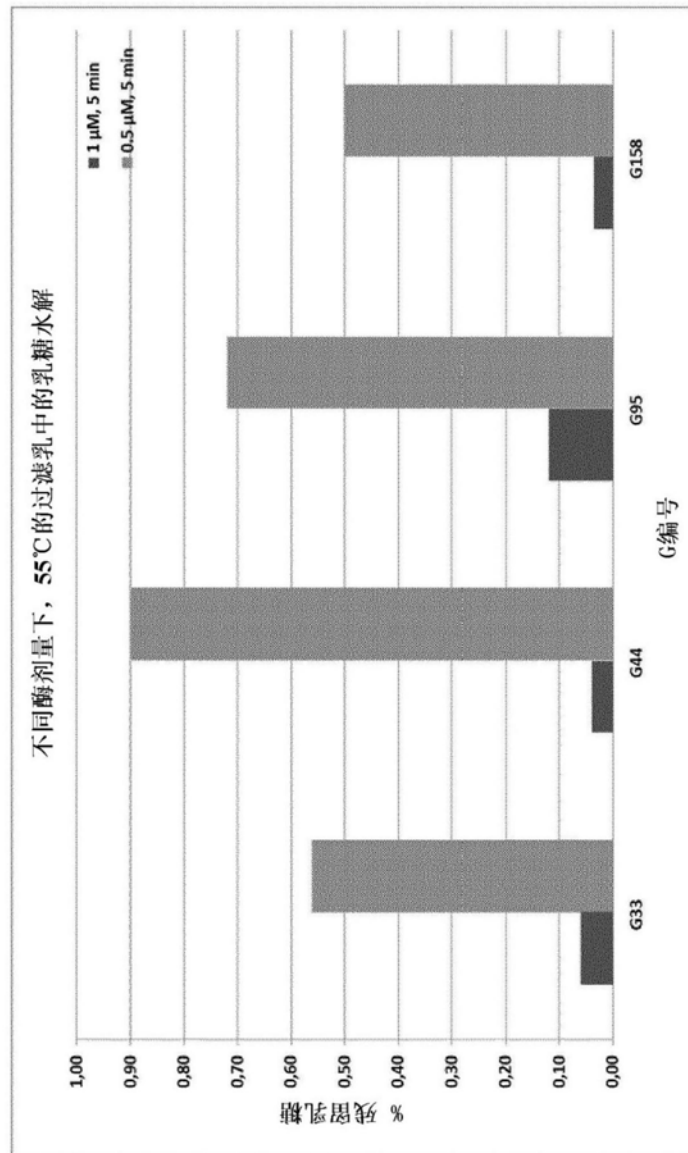


图20

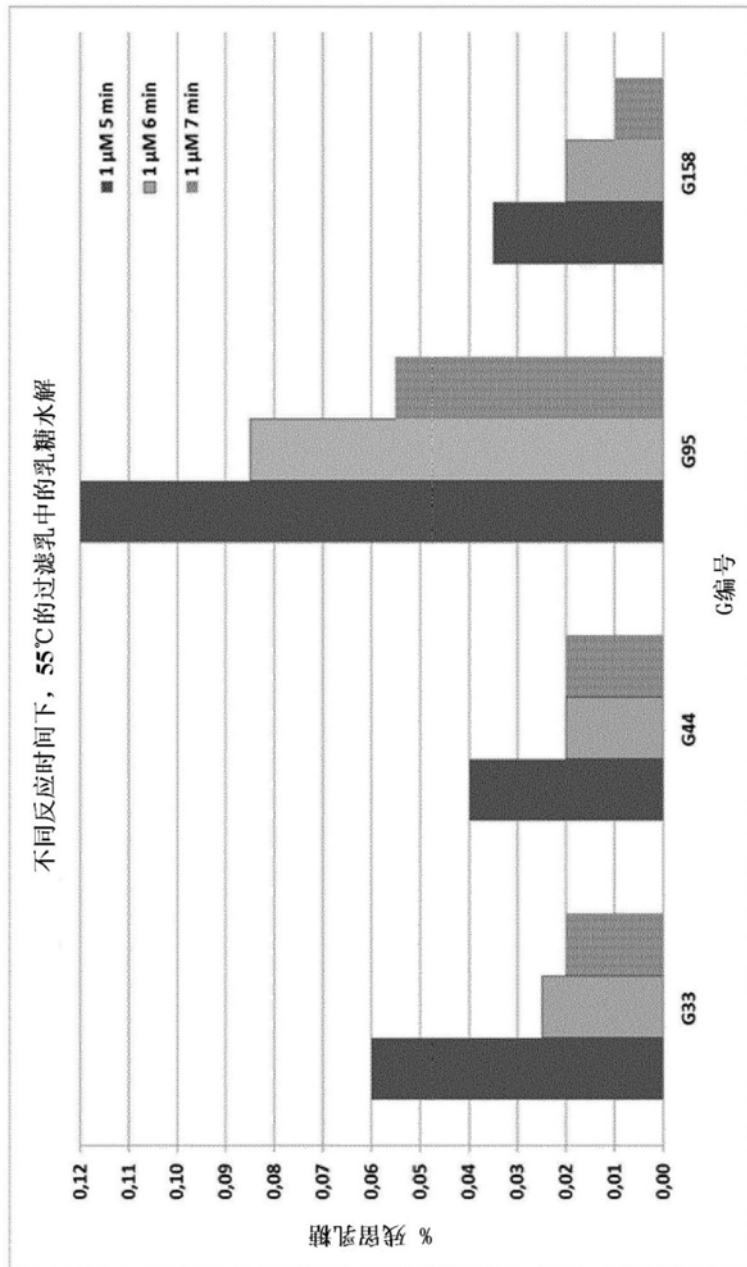


图21

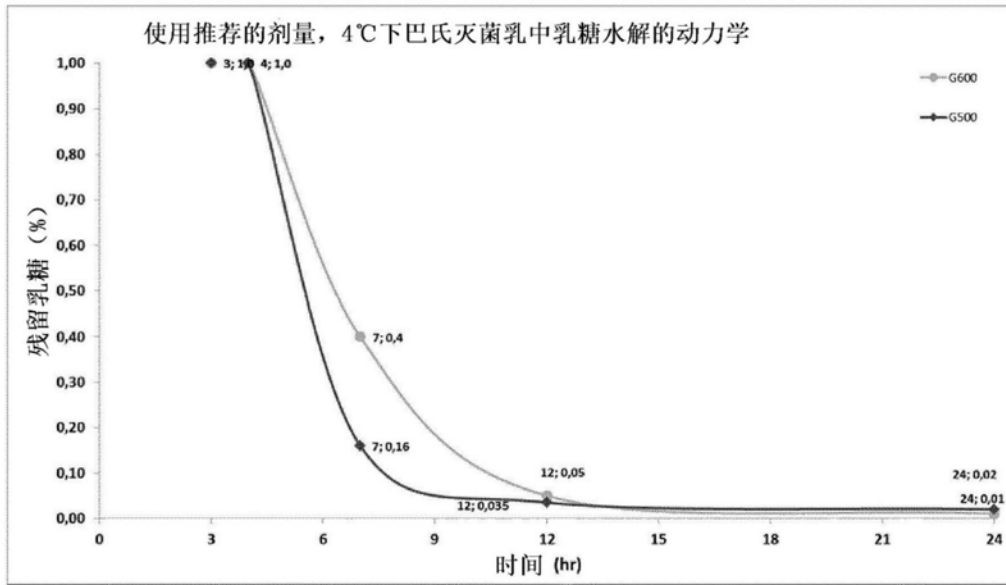


图22

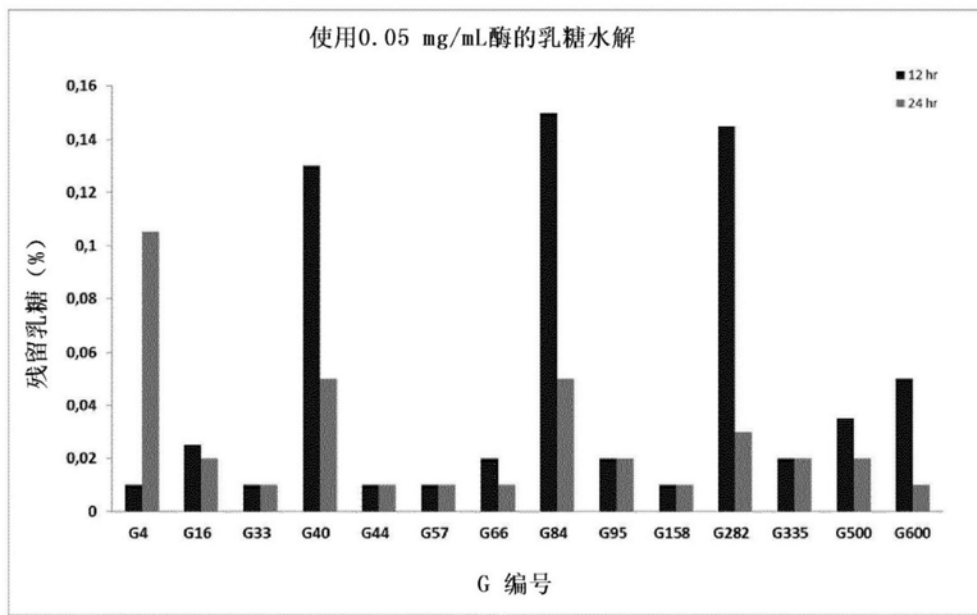


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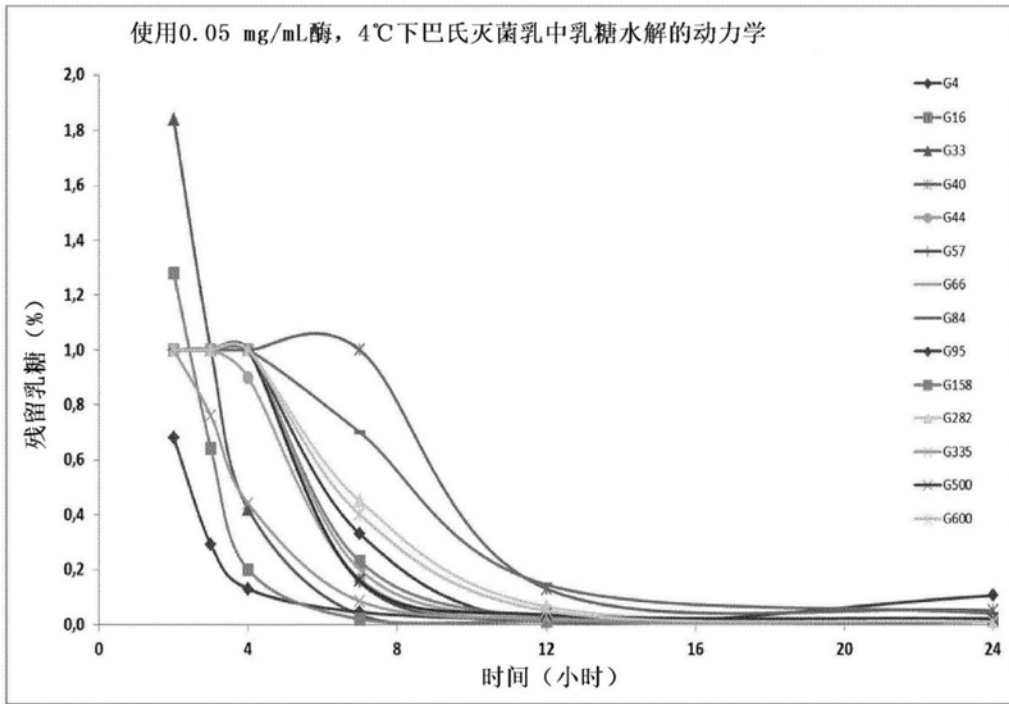


图24

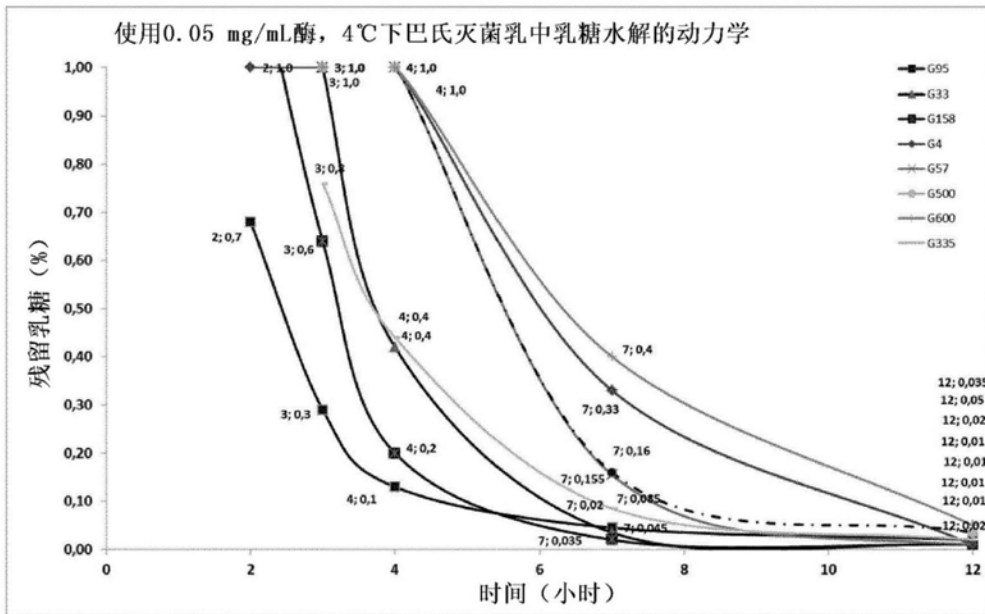


图25

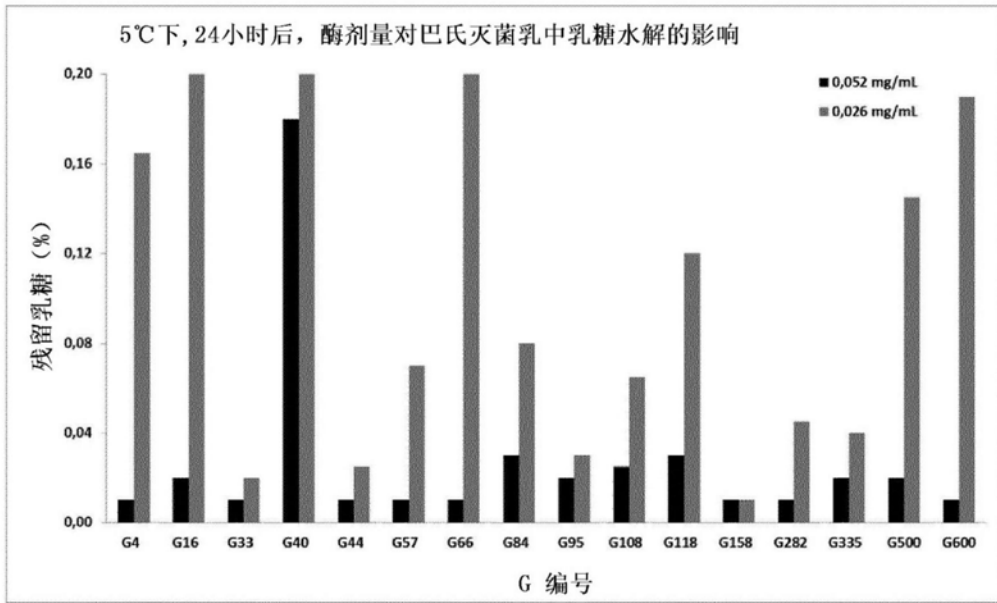


图26

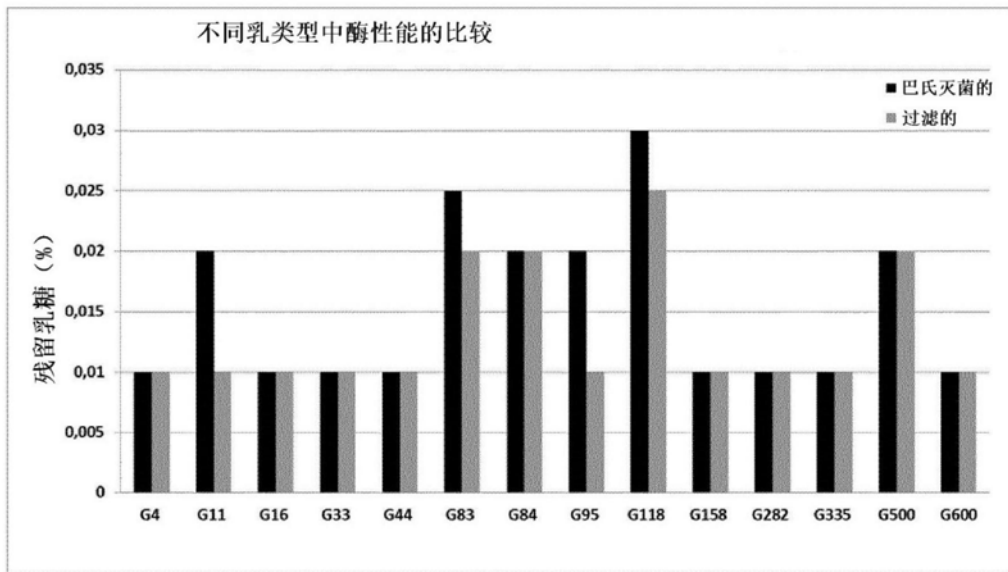


图27

G 编号	pH 6.7 4°C	pH 6,7 37°C	pH 6,7 43°C	% 半乳糖抑制
4	9,4	118,1	84,7	34
11	8,4	69,2	111,3	9
16	1,6	23,4	17,0	45
33	12,5	130,1	173,3	3
35	12,5	121,0	100,9	27
40	1,2	15,8	12,4	53
44	24,2	331,5	295,9	35
51	20,7	250,6	214,3	35
57	7,4	104,6	97,2	47
62	5,2	48,5	37,6	83
66	15,2	187,2	136,8	23
83	26,9	272,9	195,1	37
84	15,9	161,9	118,0	31
95	28,8	288,1	250,7	37
100	27,9	339,9	238,1	39
104	12,9	90,5	112,9	1
108	27,2	277,9	213,1	34
109	25,3	300,1	218,3	30
118	16,9	113,8	122,3	14
145	2,4	24,1	22,6	64
158	34,2	254,7	334,8	27
224	11,2	389,9	131,4	48
256	15,5	111,3	112,8	14
282	8,4	58,5	48,6	62
335	4,4	42,4	30,9	50
500	3,9	46,9	13,1	35
600	7,4	61,9	45,6	44

图28

G 编号	pH 5.5 4°C	pH 5.5 37°C	pH 5.5 43°C
4	1,4	44,1	29,6
11	7,6	57,9	75,5
16	0,2	0,7	0,4
33	10,4	88,2	89,9
35	1,7	51,3	40,9
40	3,3	21,8	15,7
44	4,9	111,2	80,7
51	4,0	84,6	58,8
57	0,6	17,8	13,1
62	6,0	60,5	49,7
66	0,8	63,3	42,7
83	6,7	108,4	57,2
84	8,4	99,4	62,4
95	7,0	121,3	64,7
100	5,9	128,2	55,2
104	10,6	60,4	60,5
108	7,0	116,5	69,5
109	5,7	116,5	62,6
118	14,8	76,0	65,0
145	4,9	28,8	15,8
158	18,4	129,4	126,8
224	4,4	21,0	7,4
256	14,0	62,7	57,0
282	6,4	50,8	24,9
335	0,3	9,4	3,1
500	0,1	0,4	0,5
600	4,0	32,7	26,9

图29

G 编号	pH 4.5 4°C	pH 4.5 37°C	pH 4.5 43°C
4	1,2	2,9	2,0
11	1,7	18,9	3,3
16	0,1	0	0
33	1,6	24,7	0,7
35	1,6	3,7	2,5
40	3,4	12,9	10,6
44	4,3	12,5	11,8
51	3,9	12,8	11,0
57	0,4	0,1	-0,5
62	4,2	19,9	16,6
66	0,8	1,6	1,2
83	5,6	11,2	12,4
84	7,9	22,7	17,1
95	7,0	12,8	10,4
100	5,6	12,5	11,4
104	4,5	29,7	24,1
108	6,7	14,3	12,8
109	5,5	10,4	19,8
118	5,8	37,5	25,6
145	5,0	8,9	7,2
158	4,2	12,7	25,1
224	4,1	0,0	0,0
256	7,9	23,2	17,2
282	6,3	7,5	7,2
335	0,3	0	0,0
500	0,0	0	0
600	1,6	12,2	5,0

图30