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(73)专利权人 科汉森母乳低聚糖股份有限公司
地址 德国莱茵布赖特巴赫
(72)发明人 S·詹尼温 D·瓦滕伯格
(74)专利代理机构 北京市立方律师事务所 11330
专利代理师 李娜
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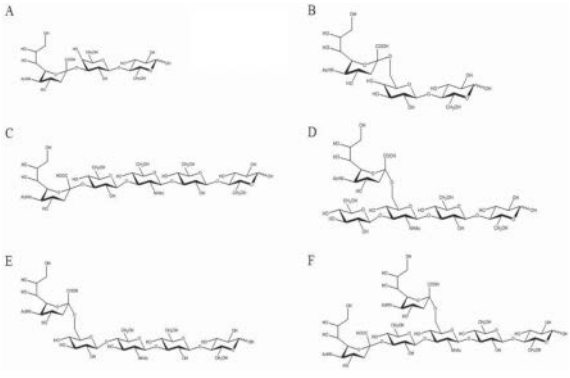
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(54)发明名称

唾液酸转移酶及其在生产唾液酸化低聚糖中的用途

(57)摘要

本申请公开了用于生产唾液酸化低聚糖的方法、遗传工程化的细胞、唾液酸转移酶和编码所述唾液酸转移酶的核酸分子,以及所述唾液酸化低聚糖用于提供营养组合物的用途。




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1.一种用于生产唾液酸化低聚糖的方法,该方法包括以下步骤:

a)提供至少一种遗传工程化的细胞,其包含一种异源的唾液酸转移酶,所述异源的唾液酸转移酶用于将唾液酸残基从作为供体底物的其核苷酸激活的形式转移到受体分子中,其中所述受体分子为乳糖和/或LNT,

其中所述异源的唾液酸转移酶具有α-2,3-唾液酸转移酶活性,并且由SEQ ID NO:40、46-47、51和53-60中任一项的氨基酸序列构成;

所述异源的唾液酸转移酶具有α-2,6-唾液酸转移酶活性,并且由SEQ ID NO:65的氨基酸序列构成;或者

所述异源的唾液酸转移酶具有α-2,3-唾液酸转移酶活性和α-2,6-唾液酸转移酶活性,并且由SEQ ID NO:48的氨基酸序列构成;

b)在发酵液中并在允许生产所述唾液酸化低聚糖的条件下培育所述至少一种遗传工程化的细胞;以及任选地

c)回收所述唾液酸化低聚糖。

2.根据权利要求1所述的方法,其中所述唾液酸化低聚糖选自3'-唾液酸乳糖、6'-唾液酸乳糖、LST-a、LST-b和DSLNT。

3.根据权利要求1所述的方法,其中所述发酵液中包含至少一种碳源,所述至少一种碳源选自葡萄糖、果糖、蔗糖、甘油及其组合。

4.根据权利要求1所述的方法,其中所述发酵液中包含选自N-乙酰葡糖胺、半乳糖和唾液酸中的至少一种。

5.根据权利要求1所述的方法,其中所述至少一种遗传工程化的细胞是在不存在和/或不添加选自N-乙酰葡糖胺、半乳糖和唾液酸中的一种或多种的情况下培养的。

6.根据权利要求1所述的方法,其中所述至少一种遗传工程化的细胞是在存在选自乳糖和LNT中的至少一种的情况下培养的。

7.根据权利要求1所述的方法,其中所述的遗传工程化的细胞包含含有编码异源的唾液酸转移酶的核苷酸序列的核酸分子,所述核苷酸序列选自:

i.SEQ ID NO:7、13-15、18、20-27和32中任一项所代表的核苷酸序列;和

ii.编码SEQ ID NO:40、46-48、51、53-60和65中任一项所代表的多肽的核苷酸序列,

其中所述核苷酸序列可操作地连接到至少一种核酸表达控制序列,所述核酸表达控制序列影响在遗传工程化的细胞中所述编码异源的唾液酸转移酶的核苷酸序列的转录和/或翻译。

8.根据权利要求1所述的方法,其中与遗传工程化前的细胞相比,所述的遗传工程化的细胞具有选自CMP-N-乙酰神经氨酸、UDP-N-乙酰葡糖胺、UDP-半乳糖和GDP-岩藻糖中的一种或多种核苷酸激活的糖的增加的产量。

9.根据权利要求1所述的方法,其中与遗传工程化前的细胞相比,所述遗传工程化的细胞具有编码多肽的一种或多种基因的过表达,所述多肽能够具有选自以下的酶活性:L-谷氨酰胺:D-果糖-6-磷酸转氨酶、N-乙酰葡糖胺-1-磷酸尿苷酰转移酶、葡糖胺-1-磷酸乙酰转移酶、磷酸葡糖胺变位酶、葡糖胺-6-磷酸-N-乙酰转移酶、N-乙酰葡糖胺-2-差向异构酶、UDP-N-乙酰葡糖胺-2-差向异构酶、唾液酸合酶、磷酸烯醇丙酮酸合酶、CMP-唾液酸合成酶、UDP-半乳糖-4-差向异构酶、半乳糖-1-磷酸尿苷基转移酶、磷酸葡萄糖变位酶、葡萄糖-1-

磷酸尿苷基转移酶、磷酸甘露糖变位酶、甘露糖-1-磷酸鸟苷酰转移酶、GDP-甘露糖4,6-脱水酶、GDP-L-岩藻糖合酶和岩藻糖激酶/L-岩藻糖-1-磷酸-鸟苷转移酶。

10.根据权利要求1所述的方法,其中与遗传工程化前的细胞相比,该细胞缺乏选自以下的一种或多种酶活性或具有降低的选自以下的一种或多种酶活性:β-半乳糖苷酶、葡糖胺-6-磷酸脱氨酶、N-乙酰葡糖胺-6-磷酸脱乙酰酶、N-乙酰甘露糖胺激酶、N-乙酰甘露糖胺-6-磷酸差向异构酶和N-乙酰神经氨酸醛缩酶。

11.根据权利要求1所述的方法,其中编码β-半乳糖苷酶、葡糖胺-6-磷酸脱氨酶、N-乙酰葡糖胺-6-磷酸脱乙酰酶、N-乙酰甘露糖胺激酶、N-乙酰甘露糖胺-6-磷酸差向异构酶和N-乙酰神经氨酸醛缩酶的基因中的一种或多种在遗传工程化的细胞中已被缺失或其表达已被失活。

12.根据权利要求1所述的方法,其中所述至少一种遗传工程化的细胞包含选自功能性乳糖通透酶、功能性岩藻糖通透酶和功能性唾液酸输入蛋白中的至少一种。

13.根据权利要求12所述的方法,其中所述至少一种遗传工程化的细胞包含并表达至少一种核苷酸序列,该核苷酸序列编码选自功能性乳糖通透酶、功能性岩藻糖通透酶和功能性唾液酸输入蛋白中的一种。

14.根据权利要求1所述的方法,其中所述细胞具有选自以下的至少一种糖基转移酶的活性:β-1,3-N-乙酰葡糖胺基转移酶、β-1,3-半乳糖基转移酶、β-1,4-半乳糖基转移酶、α-2,3-唾液酸转移酶和α-2,6-唾液酸转移酶。

15.一种用于生产唾液酸化低聚糖的方法,所述方法包括:

a)在反应混合物中提供唾液酸转移酶,所述唾液酸转移酶用于将唾液酸残基从作为供体底物的其核苷酸激活的形式转移到受体分子中,其中所述受体分子为乳糖和/或LNT,

其中所述唾液酸转移酶具有α-2,3-唾液酸转移酶活性,并且由SEQ ID NO:40、46-47、51和53-60中任一项的氨基酸序列构成;

所述唾液酸转移酶具有α-2,6-唾液酸转移酶活性,并且由SEQ ID NO:65的氨基酸序列构成;或者

所述唾液酸转移酶具有α-2,3-唾液酸转移酶活性和α-2,6-唾液酸转移酶活性,并且由SEQ ID NO:48的氨基酸序列构成;

b)将所述唾液酸转移酶与供体底物和受体分子接触,其中所述受体分子为乳糖和/或LNT。

# 唾液酸转移酶及其在生产唾液酸化低聚糖中的用途

[0001] 本发明涉及唾液酸转移酶、其在生产唾液酸化低聚糖中的用途,以及涉及所述唾液酸化低聚糖在提供营养制剂中的用途。

## 背景技术

[0002] 到目前为止,已经鉴定出150多种结构不同的人乳低聚糖(HMO)。虽然HMO只占总人乳营养物中的少量,但在过去几十年中,它们对母乳喂养婴儿的发育的非常有益的作用变得显而易见。

[0003] 人乳中总HMO含量的最高达20%是酸性的。因此,这些HMO分子具有至少一个唾液酸部分。虽然人乳中含有的唾液酸中只有3%能够以游离形式获得,但23%和74%分别与(糖-)蛋白和低聚糖结合。唾液酸家族中最常见的成员是N-乙酰神经氨酸(Neu5Ac)。作为低聚糖的一部分,N-乙酰神经氨酸通常导致糖的生物活性。

[0004] 观察到唾液酸化的HMO(SHMO)支持对病原体的抗性和肠道的抗性。值得注意的是,最近的研究进一步证明了长链SHMO抵御坏死性小肠结肠炎——早产儿中最常见和最致命的疾病之一——的保护作用。此外,SHMO被认为支持婴儿的大脑发育及其认知能力。

[0005] 虽然不同供体之间HMO谱的广泛变化阻碍了对酸性低聚糖的绝对定量,特别是影响了唾液酸乳-N-四糖的结构异构体,但最丰富的酸性HMO为3'-唾液酸乳糖(3'-SL)、6'-唾液酸乳糖(6'-SL)、唾液酸乳-N-四糖a(LST-a)、唾液酸乳-N-四糖b(LST-b)和唾液酸乳-N-四糖c(LST-c)和二唾液酸乳-N-四糖(DSLNT)。

[0006] 关于唾液酸化HMO的结构复杂性(图1),它们的化学或(化学)酶法合成具有挑战性,并涉及许多困难,例如,立体化学的控制、特定连接的形成、原料的可用性。最后,尽管这类合成方法的集合可成功的用于一些SHMO,但它们的昂贵性和令人不满意的产量限制了用于商业目的唾液酸化HMO的成本效益生产。

[0007] 一般来说,对微生物的代谢工程化以生产HMO代表了以工业规模生产HMO的最有前途的方法,并且已经被开发用于生产2'-岩藻糖基乳糖、3-岩藻糖基乳糖和3'-唾液酸乳糖。

[0008] 然而,生产HMO的生物合成途径的工程化往往受限于参与所需HMO生物合成的糖基转移酶(例如,岩藻糖基-、半乳糖基-、N-乙酰-葡糖胺基-或唾液酸转移酶)的特异性和活性,特别是在异源表达系统(如重组细菌细胞)中时。

[0009] 不幸的是,编码人唾液酸转移酶的基因几乎不在原核微生物中表达。因此,这些基因和酶不适用于使用遗传工程化的细菌菌株(如大肠杆菌(Escherichia coli))来生产唾液酸化HMO的生物技术方法。

[0010] 到目前为止,已经鉴定和表征了几种来自细菌菌种的唾液酸转移酶(SiaT),例如来自奈瑟氏菌属(Neisseria)、弯曲杆菌属(Campylobacter)、巴氏杆菌属(Pasteurella)、螺旋杆菌属(Helicobacter)和发光杆菌属(Photobacterium)以及来自哺乳动物和病毒。根据蛋白质序列相似性,唾液酸转移酶通常被分为六个糖基转移酶(GT)家族。其中所有真核和病毒唾液酸转移酶被分组为GT家族29,而细菌SiaT则被包含在GT4、GT38、GT42、GT52或GT80组中。此外,唾液酸转移酶和多唾液酸转移酶可因它们形成的键而区分为,例如α-2,

3-、α-2,6-和α-2,8-唾液酸转移酶。所有这些唾液酸转移酶将唾液酸残基从胞苷5'-单磷酸唾液酸(例如CMP-Neu5Ac)转移到多种受体分子,通常是半乳糖-(Gal)、N-乙酰半乳糖胺(GalNAc)或N-乙酰葡糖胺(GalNAc)部分或其他唾液酸(Sia)部分。

[0011] 几种细菌唾液酸转移酶在过去已得到充分表征,并且已经被证明适合于生产3'-SL或6'-SL。人们对使得能够合成唾液酸化的五糖或六糖(如LST-a、LST-b或DSLNT)的唾液酸转移酶知之甚少,从而限制了建立用于生产这些SHMO中任何一种的生产方法。因此,无法获得高纯度的这些所需低聚糖,阻碍了对其健康有益特性的广泛科学评估。

[0012] 因此,需要一种具有成本效益的方法来大量且高纯度地生产一种或多种SHMO,特别是具有一个或两个唾液酸残基的四糖、五糖和六糖。

## 发明内容

[0013] 除其他外,本发明的目的尤其是通过鉴定和表征新的唾液酸转移酶及其在通过全细胞发酵或生物催化生产唾液酸化人乳低聚糖中的用途而得以解决的。

[0014] 根据第一方面,本申请提供了一种用于生产唾液酸化低聚糖的方法,其中一种遗传工程化细胞被用于生产所述唾液酸化低聚糖。所述遗传工程化细胞包含至少一种异源的唾液酸转移酶,其能够将唾液酸残基从供体底物转移到受体分子,其中所述受体分子选自乳糖、LNT-II和人乳低聚糖。

[0015] 根据第二方面,本申请提供了一种用于生产唾液酸化低聚糖的方法的遗传工程化细胞,其中所述遗传工程化细胞已被遗传工程化以表达异源的唾液酸转移酶,该唾液酸转移酶能够将唾液酸残基从供体底物转移到受体分子,其中所述受体分子选自乳糖、LNT-II和人乳低聚糖。

[0016] 根据第三方面,本申请提供了一种用于在细胞中增殖时表达异源的唾液酸转移酶的重组核酸分子,其中所述唾液酸转移酶能够将唾液酸残基从供体底物转移到受体分子,其中所述受体分子选自乳糖、LNT-II和人乳低聚糖。

[0017] 根据第四方面,本申请提供了能够将唾液酸残基从供体底物转移到受体分子的唾液酸转移酶,其中所述受体分子选自乳糖、LNT-II和人乳低聚糖。

[0018] 根据第五方面,本申请提供了能够将唾液酸残基从供体底物转移到受体分子的唾液酸转移酶用于生产唾液酸化低聚糖的用途,其中所述受体分子选自乳糖、LNT-II和人乳低聚糖。

[0019] 根据第六方面,本申请提供了一种通过体外生物催化生产唾液酸化低聚糖的方法,其中使用唾液酸转移酶,所述唾液酸转移酶能够将唾液酸残基从供体底物转移到受体分子,其中所述受体分子选自乳糖、LNT-II和人乳低聚糖。

[0020] 根据第七方面,本申请提供了通过根据第一方面的方法或根据第六方面的方法生产的唾液酸化低聚糖。

[0021] 根据第八方面,本申请提供了根据第七方面的唾液酸化低聚糖用于制备营养组合物的用途。

[0022] 根据第九方面,本申请提供了一种营养组合物,其含有至少一种根据第七方面的唾液酸化低聚糖。

[0023] 根据第十方面,本申请提供了一种婴儿配方物,其含有至少一种唾液酸化人乳低

聚糖。

## 附图说明

[0024] 图1描述了人乳中最丰富的酸性低聚糖的化学结构:3'-SL(A)、6'-SL(B)、LST-a(C)、LST-b(D)、LST-c(E)和DSLNT(F)。

[0025] 图2示出了过表达唾液酸转移酶基因的质粒pDEST14和pET11a的图谱。

[0026] 图3示出了由于合适的唾液酸转移酶的过表达而进行的唾液酸乳-N-四糖-a和唾液酸乳-N-四糖-b的体内生产(用箭头标记),其中描述了通过薄层色谱法对过表达siaT9或siaT19的大肠杆菌BL21(DE3)#2130细胞的胞内级分进行分离。

## 具体实施方式

[0027] 为了鉴定适用于制备唾液酸化HMO的方法的唾液酸转移酶,对核酸数据库和蛋白质数据库进行了检索。通过与已知的糖基转移酶的序列相似性,鉴定出100种推定的唾液酸转移酶。评估所述推定的唾液酸转移酶的唾液酸转移酶活性。

[0028] 根据第一方面,本申请提供了一种用于生产唾液酸化低聚糖的方法,该方法包括以下步骤:

[0029] a)提供至少一种遗传工程化细胞,其包含一种异源的唾液酸转移酶,所述异源的唾液酸转移酶能够具有α-2,3-唾液酸转移酶活性和/或α-2,6-唾液酸转移酶活性,用于将唾液酸残基从作为供体底物的CMP-激活形式转移到受体分子中,所述受体分子选自乳糖、LNT-II和人乳低聚糖;

[0030] b)在发酵液中和允许生产所述唾液酸化低聚糖的条件下,培养所述至少一种遗传工程化细胞;以及

[0031] c)回收所述唾液酸化低聚糖。

[0032] 该方法是一种用于生产唾液酸化低聚糖的方法。

[0033] 本文使用的术语"低聚糖"是指单糖残基的聚合物,其中所述聚合物包含至少三个单糖残基,但不超过10个单糖残基,优选地不超过7个单糖残基。低聚糖是单糖的线性链或者支链。此外,低聚糖的单糖残基可能具有许多化学修饰的特征。因此,低聚糖可包含一个或多个非糖部分。

[0034] 本文使用的术语"唾液酸化低聚糖"是指包含一个或多个唾液酸残基的低聚糖。在优选的实施方案中,所述唾液酸残基为N-乙酰神经氨酸(Neu5Ac)残基。该N-乙酰神经氨酸残基通常是从作为供体底物的CMP-Neu5Ac转移到受体分子。

[0035] 用于生产唾液酸化低聚糖的方法包括提供一种含有异源的唾液酸转移酶的遗传工程化细胞的步骤,该异源的唾液酸转移酶能够具有α-2,3-唾液酸转移酶活性和/或α-2,6-唾液酸转移酶活性。

[0036] 遗传工程化的细胞是原核细胞或真核细胞。优选地,所述遗传工程化细胞为微生物细胞。适当的微生物细胞包括酵母细胞、细菌细胞、古细菌细胞、藻类细胞和真菌细胞。

[0037] 在一个额外的和/或替代的实施方案中,微生物细胞为原核细胞,优选地为细菌细胞,更优选地为选自以下的细菌细胞:芽孢杆菌属(Bacillus)、乳杆菌属(Lactobacillus)、乳球菌属(Lactococcus)、肠球菌属(Enterococcus)、双歧杆菌属(Bifidobacterium)、芽胞

乳杆菌属种(Sporolactobacillus spp.)、小单孢菌属种(Micromonospora spp.)、微球菌属种(Micrococcus spp.)、红球菌属种(Rhodococcus spp.)和假单胞菌属(Pseudomonas)。适合的细菌种有枯草芽孢杆菌(Bacillus subtilis)、地衣芽孢杆菌(Bacillus licheniformis)、凝结芽孢杆菌(Bacillus coagulans)、嗜热芽孢杆菌(Bacillus thermophilus)、侧孢芽孢杆菌(Bacillus laterosporus)、巨大芽孢杆菌(Bacillus megaterium)、蕈状芽孢杆菌(Bacillus mycoides)、短小芽孢杆菌(Bacillus pumilus)、迟缓芽孢杆菌(Bacillus lentus)、蜡样芽孢杆菌(Bacillus cereus)、环状芽孢杆菌(Bacillus circulans)、长双歧杆菌(Bifidobacterium longum)、婴儿双歧杆菌(Bifidobacterium infantis)、两歧双歧杆菌(Bifidobacterium bifidum)、弗氏柠檬酸杆菌(Citrobacter freundii)、解纤维素梭菌(Clostridium cellulolyticum)、杨氏梭菌(Clostridium ljungdahlii)、自产乙醇梭菌(Clostridium autoethanogenum)、丙酮丁醇梭菌(Clostridium acetobutylicum)、谷氨酸棒状杆菌(Corynebacterium glutamicum)、屎肠球菌(Enterococcus faecium)、嗜热肠球菌(Enterococcus thermo philes)、大肠杆菌、草生欧文氏菌(Erwinia herbicola)(成团泛菌(P antoea agglomerans))、嗜酸乳杆菌(Lactobacillus acidophilus)、唾液乳杆菌(Lactobacillus salivarius)、植物乳杆菌(Lactobacillus plantarum)、瑞士乳杆菌(Lactobacillus helveticus)、德氏乳杆菌(Lactobacillus delbrueckii)、鼠李糖乳杆菌(Lactobacillus rhamnosus)、保加利亚乳杆菌(Lactobacillus bulgaricus)、卷曲乳杆菌(Lactobacillus crispatus)、加氏乳杆菌(Lactobacillus gasseri)、干酪乳杆菌(Lactobacillus casei)、罗伊氏乳杆菌(Lactobacillus reuteri)、詹氏乳杆菌(Lacto bacillus jensenii)、乳酸乳球菌(Lactococcus lactis)、柠檬泛菌(Pantoea citrea)、胡萝卜软腐果胶杆菌(Pectobacterium carotovorum)、费氏丙酸杆菌(Proprionibacterium freudenreichii)、荧光假单胞菌(Pseu domonas fluorescens)、铜绿假单胞菌(Pseudomonas aeruginosa)、嗜热链球菌(Streptococcus thermophiles)和野油菜黄单胞菌(Xanthomon as campestris)。

[0038] 在替代的实施方案中,真核细胞是酵母细胞、昆虫细胞、植物细胞或哺乳动物细胞。酵母细胞优选地选自酵母属种(Saccharomyces sp.)(特别是酿酒酵母(Saccharomyces cerevisiae))、复膜孢酵母属种(Sacch aromycopsis sp.)、毕赤酵母属种(Pichia sp.)(特别是巴斯德毕赤酵母(Pichia pastoris))、汉森酵母属种(Hansenula sp.)、克鲁维酵母属种(Kluyveromyces sp.)、亚罗酵母属种(Yarrowia sp.)、红酵母属种(Rhodotorula sp.)和裂殖酵母属种(Schizosaccharomyces sp.)。

[0039] 遗传工程化的细胞已被遗传工程化以包含异源的唾液酸转移酶。

[0040] 本文使用的术语“遗传工程化”是指使用分子生物学方法进行的对细胞的遗传组成的修饰。细胞遗传组成的修饰可能包括基因在物种边界内部和/或跨越物种边界的转移,插入、缺失、替换和/或修饰核苷酸、三联体、基因、开放阅读框、启动子、增强子、终止子和其他介导和/或控制基因表达的核苷酸序列。细胞的遗传组成的修饰目的是产生具有特定的、所需特性的遗传工程化的生物体。遗传工程化的细胞可以包含一个或多个在天然(非遗传工程化的)形式的细胞中不存在的基因。本领域技术人员已知用于引入外源核酸分子和/或插入外源核酸分子(重组、异源)到细胞的遗传信息中来插入、缺失或改变细胞遗传信息的

核甘酸序列的技术。遗传工程化的细胞可以包含一个或多个存在于天然形式的细胞中的基因,其中所述基因通过人工手段而被修饰并重新导入细胞。术语“遗传工程化”还包括含有这样的核酸分子的细胞,即所述核酸分子对于细胞而言是内源性的,并且已经被修饰而没有将核酸分子从细胞中移除。这些修饰包括通过基因替换、位点特异性突变和相关技术获得的修饰。

[0041] 遗传工程化的细胞包含一种异源的唾液酸转移酶。

[0042] 本文使用的术语“唾液酸转移酶”是指能够具有唾液酸转移酶活性的多肽。“唾液酸转移酶活性”是指将唾液酸残基,优选N-乙酰神经氨酸(Neu5Ac)残基,从供体底物转移到受体分子。术语“唾液酸转移酶”包含本文记载的唾液酸转移酶的功能性片段、本文记载的唾液酸转移酶的功能性变体和所述功能性变体的功能性片段。在这方面,“功能性”是指所述片段和/或变体具有唾液酸转移酶活性。唾液酸转移酶的功能性片段包括由其天然存在的基因编码的唾液酸转移酶的截短形式,该截短形式能够具有唾液酸转移酶活性。截短形式的实例为不包含所谓的前导序列的唾液酸转移酶,所述前导序列通常将多肽引导到特定的亚细胞位置。通常,这种前导序列在多肽的亚细胞转运过程中被从多肽中移除,并且在天然存在的成熟唾液酸转移酶中也不存在。

[0043] 异源的唾液酸转移酶能够将唾液酸残基从供体底物转移到受体分子。用于异源的唾液酸转移酶的术语“能够”是指异源的唾液酸转移酶的唾液酸转移酶活性,并且条件是异源的唾液酸转移酶需要适当的反应条件以具有酶活性。在没有合适的反应条件的情况下,异源的唾液酸转移酶不具有其酶活性,但是保留其酶活性,并且当合适的反应条件恢复时具有酶活性。合适的反应条件包括:存在合适的供体底物,存在合适的受体分子,存在基本辅助因子例如单价或二价离子、处于适当范围内的pH值、合适的温度等。没有必要满足影响异源的唾液酸转移酶酶反应的每个因素的最佳值,但反应条件必须使得异源的唾液酸转移酶发挥其酶活性。因此,术语“能够”排除使异源的唾液酸转移酶的酶活性受到不可逆损害的任何条件,还排除将异源的唾液酸转移酶暴露于任何这样的条件。相反,“能够”是指唾液酸转移酶具有酶活性,也就是说,如果向唾液酸转移酶提供了允许的反应条件(其中所有要求都是唾液酸转移酶实施其酶活性所必需的),即具有其唾液酸转移酶活性。

[0044] 唾液转移酶可以根据它们形成的糖键类型来区分。本文使用的术语“α-2,3-唾液酸转移酶”和“α-2,3-唾液酸转移酶活性”是指向受体分子的半乳糖或半乳糖残基添加具有α-2,3键的唾液酸残基。同样,术语“α-2,6-唾液酸转移酶”和“α-2,6-唾液酸转移酶活性”是指多肽及其酶活性,其向受体分子的半乳糖、N-乙酰半乳糖胺和/或N-乙酰葡糖胺、半乳糖残基或N-乙酰半乳糖胺残基和/或N-乙酰葡糖胺残基添加具有α-2,6键的唾液酸残基。同样,术语“α-2,8-唾液酸转移酶”和“α-2,8-唾液酸转移酶活性”是指多肽及其酶活性,其向受体分子的半乳糖、N-乙酰半乳糖胺和/或N-乙酰葡糖胺、半乳糖残基或N-乙酰半乳糖胺残基和/或N-乙酰葡糖胺残基添加具有α-2,8键的唾液酸残基。

[0045] 本文使用的术语“异源”是指对细胞或生物体来说是外源的多肽、氨基酸序列、核酸分子或核苷酸序列,即非天然存在于所述细胞或生物体中的多肽、氨基酸序列、核酸分子或核苷酸序列。本文使用的“异源序列”或“异源核酸”或“异源多肽”是源自对特定宿主细胞来说是外源的来源(例如来自不同物种),或如果来自同一来源,但从其原始形式被修饰。因此,可操作地连接到启动子的异源核酸来自不同于该启动子的来源的来源,或者,如果来自

同一来源,则从其原始形式被修饰。所述异源序列可以例如通过转染、转化、缀合或转导被稳定地导入宿主微生物宿主细胞的基因组中,从而代表一种遗传修饰的宿主细胞。可以应用技术,这些技术将取决于将要引入序列的宿主细胞。各种技术是本领域技术人员已知的并且例如公开于Sambrook et al.,Molecular Cloning:A Laboratory Manual,2nd Ed.,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,N.Y.(1989)。因此,“异源多肽”是一种非天然存在于细胞中的多肽,以及“异源的唾液酸转移酶”是一种非天然存在于细胞中的唾液酸转移酶。

[0046] 异源的唾液酸转移酶能够将唾液酸残基,例如N-乙酰神经氨酸(Neu5Ac)残基,从供体底物(例如,CMP-Neu5Ac)转移到受体分子。受体分子是乳糖、乳-N-三糖II(LNT-II)或选自人乳低聚糖的低聚糖。

[0047] 在一个额外的和/或替代的实施方案中,受体分子为选自三糖、四糖和五糖的人乳低聚糖。

[0048] 在一个额外的和/或替代的实施方案中,受体分子为选自乳-N-四糖、乳-N-新四糖、LST-a和LST-b的人乳低聚糖。

[0049] 在一个实施方案中,异源的唾液酸转移酶选自:

[0050] I.包含SEQ ID NO:1至33中任一项所代表的氨基酸序列或由其组成的多肽;

[0051] II.包含与SEQ ID NO:1至33中任一项所代表的任一种氨基酸序列具有至少80%的序列相似性的氨基酸序列或由其组成的多肽;和

[0052] III.I和II中任一种多肽的片段。

[0053] 在一个额外的和/或替代的实施方案中,遗传工程化的细胞已被转化以包含核酸分子,该核酸分子包含编码异源的唾液酸转移酶的核苷酸序列。优选地,所述核苷酸序列选自:

[0054] i.编码SEQ ID NO:1至33中任一项所代表的多肽的核苷酸序列;

[0055] ii.SEQ ID NO:34至66中任一项所代表的核苷酸序列;

[0056] iii.与编码SEQ ID NO:1至33中任一项所代表的多肽的一种核苷酸序列具有至少80%的序列相似性的核苷酸序列;

[0057] iv.与SEQ ID NO:34至66中所代表的任一种核苷酸序列具有至少80%的序列相似性的核苷酸序列;

[0058] v.与i、ii、iii和iv中任一种核苷酸序列互补的核苷酸序列;和

[0059] vi.i、ii、iii、iv和v中任一种核苷酸序列的片段。

[0060] 表述“SEQ ID NO:1至33中任一项”是指SEQ ID NO:1、SEQ ID NO:2、SEQ ID NO:3、SEQ ID NO:4、SEQ ID NO:5、SEQ ID NO:6、SEQ ID NO:7、SEQ ID NO:8、SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:1 1、SEQ ID NO:12、SEQ ID NO:13、SEQ ID NO:14、SEQ ID NO:15、SEQ ID NO:16、SEQ ID NO:17、SEQ ID NO:18、SEQ ID NO:19、SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO:22、SEQ ID NO:23、SEQ ID NO:24、SEQ ID NO:25、SEQ ID NO:26、SEQ ID NO:27、SEQ ID NO:28、SEQ ID NO:29、SEQ ID NO:30、SEQ ID NO:31、SEQ ID NO:32和SEQ ID NO:33中的任一项。同样的原则适用于表述“SEQ ID NO:34至66中任一项”。一般来说,表述“SEQ ID NO:X至Z中的任一项”,其中“X”和“Z”代表一个自然数,是指包含X至Z的识别号的“SEQ ID NO”中的任一项所代表的所有序列(核苷酸序列或氨基酸序列)。

[0061] 此外,遗传工程化的细胞已经被遗传工程化来表达编码异源的唾液酸转移酶的核苷酸序列。为此,编码异源的唾液酸转移酶的核苷酸序列可操作地连接到至少一个表达控制序列上,所述表达控制序列影响遗传工程化的细胞中所述编码异源的唾液酸转移酶的核苷酸序列的转录和/或翻译。

[0062] 本文使用的术语“可操作地连接”是指编码异源的唾液酸转移酶的核苷酸序列与第二核苷酸序列——核酸表达控制序列(如启动子、操纵子、增强子、调控子、转录因子结合位点阵列、转录终止子、核糖体结合位点)之间的功能性连接,其中所述表达控制序列影响对应于编码异源的唾液酸转移酶的核苷酸序列的核酸的转录和/或翻译。因此,术语“启动子”指的是DNA聚合物中通常位于基因“前面”并提供转录成mRNA的起始位点的DNA序列。“调控子”DNA序列,通常也是在给定的DNA聚合物中的基因“上游”(即前面),结合确定转录起始频率(或速率)的蛋白质。这些序列统称为“启动子/调控子”或“控制”DNA序列,它们在功能DNA聚合物的一个选定基因(或一系列基因)之前,相互配合以确定基因的转录(和最终表达)是否会发生。在DNA聚合物中“跟随”基因并提供向mRNA的转录的终止信号的DNA序列被称为转录“终止子”序列。

[0063] 在一个实施方案中,遗传工程化的细胞包含能够具有α-2,3-唾液酸转移酶活性的异源的唾液酸转移酶,并且所述人乳低聚糖为LNT。由此产生的唾液酸低聚糖为LST-a。

[0064] 在一个额外的和/或替代的实施方案中,能够具有α-2,3-唾液酸转移酶活性的异源的唾液酸转移酶选自:

[0065] I.包含SEQ ID NO:1至27中任一项所代表的氨基酸序列或由其组成的多肽;

[0066] II.包含与SEQ ID NO:1至27中任一项所代表的任一种氨基酸序列具有至少80%相似性的氨基酸序列或由其组成的多肽;和

[0067] III.I和II中任一种多肽的片段。

[0068] 在一个额外的和/或替代的实施方案中,遗传工程化的细胞包含重组或合成核酸分子,该分子包含编码所述异源的唾液酸转移酶的至少一种核苷酸序列,所述异源的唾液酸转移酶能够具有α-2,3-唾液酸转移酶活性,其中所述至少一种核苷酸序列选自:

[0069] i.编码SEQ ID NO:1至27中任一项所代表的多肽的核苷酸序列;

[0070] ii.SEQ ID NO:34至60中任一项所代表的核苷酸序列;

[0071] iii.与编码SEQ ID NO:1至27中任一项所代表的多肽的一种核苷酸序列具有至少80%的序列相似性的核苷酸序列;

[0072] iv.与SEQ ID NO:34至60中所代表的任一种核苷酸序列具有至少80%的序列相似性的核苷酸序列;

[0073] v.与i、ii、iii和iv中任一种核苷酸序列互补的核苷酸序列;和

[0074] vi.i、ii、iii、iv和v中任一种核苷酸序列的片段。

[0075] 在一个额外的和/或替代的实施方案中,通过根据如实施例5所述的方法使用LC-MS/MS对LNT唾液酸化进行的定量分析,与SEQ ID NO:27所代表的SiaT16的相对效力相比,能够具有α-2,3-唾液酸转移酶活性的异源的唾液酸转移酶具有至少100倍、至少200倍、至少300倍、至少1000倍、至少10000倍的相对效力。

[0076] 在另一个实施方案中,所述异源的唾液酸转移酶能够具有α-2,6-唾液酸转移酶活性,以及所述人乳低聚糖为LNT。由此产生的唾液酸化低聚糖为LST-b。

[0077] 在另一个实施方案中,能够具有α-2,6-唾液酸转移酶活性的异源的唾液酸转移酶选自:

[0078] I.包含SEQ ID NO:28至33中任一项所代表的氨基酸序列或由其组成的多肽;

[0079] II.包含与SEQ ID NO:28至33中任一项所代表的任一种氨基酸序列具有至少80%相似性的氨基酸序列或由其组成的多肽;和

[0080] III.I和II中任一种多肽的片段。

[0081] 在一个额外的和/或替代的实施方案中,遗传工程化的细胞包含重组或合成的核酸分子,该分子包含至少一种编码所述异源的唾液酸转移酶的核苷酸序列,所述异源的唾液酸转移酶能够具有α-2,6-唾液酸转移酶活性,其中所述至少一种核苷酸序列选自:

[0082] i.编码SEQ ID NO:28至33中任一项所代表的多肽的核苷酸序列;

[0083] ii.SEQ ID NO:61至66中任一项所代表的核苷酸序列;

[0084] iii.与编码SEQ ID NO:28至33中任一项所代表的多肽的一种核苷酸序列具有至少80%的序列相似性的核苷酸序列;

[0085] iv.与SEQ ID NO:61至66中所代表的任一种核苷酸序列具有至少80%的序列相似性的核苷酸序列;

[0086] v.与i、ii、iii和iv中任一种核苷酸序列互补的核苷酸序列;和

[0087] vi.i、ii、iii、iv和v中任一种核苷酸序列的片段。

[0088] 在一个额外的和/或替代的实施方案中,通过根据如实施例5所述的方法使用LC-MS/MS对LNT唾液酸化进行的定量分析,与SEQ ID NO:33所代表的SiaT5的相对效力相比,能够具有α-2,3-唾液酸转移酶活性的异源的唾液酸转移酶具有至少100倍、更优选至少200倍、最优选至少300倍的相对效力。

[0089] 在一个额外的和/或替代的实施方案中,至少一种遗传工程化的细胞具有选自CMP-N-乙酰神经氨酸、UDP-N-乙酰葡糖胺、UDP-半乳糖和GDP-岩藻糖的一种或多种核苷酸激活的糖的增加的产量。优选地,所述至少一种遗传工程化的细胞已被进一步遗传工程化,以具有一种或多种所述核苷酸激活的糖的增加的产量。与进一步遗传工程化以具有至少一种所述核苷酸激活的糖的增加的产量之前的进一步遗传工程化的细胞的祖细胞中相同的核苷酸激活的糖的产量相比,在进一步遗传工程化的细胞中至少一种所述核苷酸激活的糖的产量增加。

[0090] 在一个额外的和/或替代的实施方案中,至少一种细胞经过进一步遗传工程化以过表达编码多肽的一种或多种基因,该多肽能够具有选自以下的酶活性:L-谷氨酰胺:D-果糖-6-磷酸转氨酶、N-乙酰葡糖胺-1-磷酸尿苷酰转移酶(N-acetylglucosamine-1-phosphate uridyltransferase)、葡糖胺-1-磷酸乙酰转移酶、磷酸葡糖胺变位酶、葡糖胺-6-磷酸-N-乙酰转移酶、N-乙酰葡糖胺-2-差向异构酶、UDP-N-乙酰葡糖胺-2-差向异构酶、唾液酸合酶、磷酸烯醇丙酮酸合酶、CMP-唾液酸合成酶、UDP-半乳糖-4-差向异构酶、半乳糖-1-磷酸尿苷基转移酶(galactose-1-phosphate uridylyltransferase)、磷酸葡萄糖变位酶、葡萄糖-1-磷酸尿苷基转移酶(glucose-1-phosphae uridylyltransferase)、磷酸甘露糖变位酶、甘露糖-1-磷酸鸟苷酰转移酶(mannose-1-phosphate guanosyltransferase)、GDP-甘露糖-4,6-脱水酶、GDP-L-岩藻糖合酶和岩藻糖激酶/L-岩藻糖-1-磷酸-鸟苷转移酶(fucosekinase/L-fucose-1-phosphate-guanyltransferase)。

所述一种或多种基因的过表达是,与在进一步遗传工程化以具有所述一种或多种基因的过表达之前的进一步遗传工程化的细胞的祖细胞相比的过表达。

[0091] 一种或多种所述基因的过表达增加了遗传工程化的细胞中对应酶的量,从而提高了细胞中对应的酶活性,以增加至少一种所述核苷酸激活的糖的细胞内产量。

[0092] 在一个额外的和/或替代的实施方案中,与遗传工程化前的细胞相比,至少一种遗传工程化的细胞缺乏或具有选自以下的一种或多种酶活性降低的活性:β-半乳糖苷酶活性、葡糖胺-6-磷酸脱氨酶、N-乙酰葡糖胺-6-磷酸脱乙酰酶、N-乙酰甘露糖胺激酶、N-乙酰甘露糖胺-6-磷酸差向异构酶和N-乙酰神经氨酸醛缩酶。

[0093] 在一个额外的和/或替代的实施方案中,通过对细胞的进一步细胞遗传工程化,编码β-半乳糖苷酶、葡糖胺-6-磷酸脱氨酶、N-乙酰葡糖胺-6-磷酸脱乙酰酶、N-乙酰甘露糖胺激酶、N-乙酰甘露糖胺-6-磷酸差向异构酶和N-乙酰神经氨酸醛缩酶中的一种或多种基因已经被从遗传工程化的细胞的基因组中缺失,或编码β-半乳糖苷酶、葡糖胺-6-磷酸脱氨酶、N-乙酰葡糖胺-6-磷酸脱乙酰酶、N-乙酰甘露糖胺激酶、N-乙酰甘露糖胺-6-磷酸差向异构酶和N-乙酰神经氨酸醛缩酶的一种或多种基因的表达已经在遗传工程化的细胞中被失活或至少降低。与在进一步遗传工程化以具有所述基因的降低的表达之前的进一步遗传工程化的细胞的祖细胞相比,所述基因的表达在进一步遗传工程化的细胞中被降低。

[0094] 在一个额外的和/或替代的实施方案中,至少一种遗传工程化的细胞包含选自功能性乳糖通透酶、功能性岩藻糖通透酶和功能性唾液酸转运蛋白(输入蛋白)(transporter(importer))的至少一种,优选地包含并表达至少一种编码选自功能性乳糖通透酶、功能性岩藻糖通透酶和功能性唾液酸转运蛋白(输入蛋白)中的一种的核苷酸序列。

[0095] 在一个额外的和/或替代的实施方案中,遗传工程化的细胞具有至少一种糖基转移酶的活性,所述糖基转移酶选自β-1,3-N-乙酰葡糖胺基转移酶、β-1,3-半乳糖基转移酶、β-1,4-半乳糖基转移酶、α-2,3-唾液酸转移酶和α-2,6-唾液酸转移酶。

[0096] 在一个额外的和/或替代的实施方案中,至少一种遗传工程化的细胞在发酵液中并在允许生产唾液酸化低聚糖的条件下培养。

[0097] 所述发酵液中包含至少一种用于遗传工程化的细胞的碳源。所述至少一种碳源优选地选自葡萄糖、果糖、蔗糖、甘油及其组合。

[0098] 在一个额外的和/或替代的实施方案中,发酵液包含选自N-乙酰葡糖胺、半乳糖和唾液酸中的至少一种。

[0099] 在一个额外的和/或替代的实施方案中,其中至少一种遗传工程化的细胞是在没有和/或不添加选自N-乙酰葡糖胺、半乳糖和唾液酸的一种或多种的情况下培养的,所述至少一种遗传工程化的细胞是在存在乳糖、乳酸-N-三糖II(LNT-II)或至少一种HMO(优选地选自三糖、四糖和五糖的HMO,更优选地选自LNT和LNnT)的情况下培养的。

[0100] 所述方法包含回收由至少一种遗传工程化的细胞在其发酵液培养过程中生产的唾液酸化低聚糖的任选步骤。在已移除遗传工程化的细胞后,可以从发酵液中回收唾液酸化低聚糖,例如通过离心,和/或可以从细胞中回收,例如通过离心从发酵液中收获细胞,并进行细胞裂解步骤。随后,唾液酸化低聚糖可以通过本领域技术人员已知的适合的技术从发酵液和/或细胞裂解产物中进一步纯化。适合的技术包括微滤、超滤、透滤、模拟移动床型色谱、电透析、反渗透、凝胶过滤、阴离子交换色谱、阳离子交换色谱等。

[0101] 根据第二方面,本申请提供了一种遗传工程化的细胞,用于生产唾液酸化低聚糖的方法中。在本文之前已经记载了与该方法有关的所述遗传工程化的细胞和所述遗传工程化的细胞的优选实施方案。因此,遗传工程化的细胞包含异源的唾液酸转移酶,所述异源的唾液酸转移酶能够具有α-2,3-唾液酸转移酶活性和/或α-2,6-唾液酸转移酶活性,以将唾液酸残基(例如N-乙酰神经氨酸(Neu5Ac)残基)从作为供体底物的核苷酸激活的形式(例如CMP-Neu5Ac)转移到受体分子,其中受体分子选自乳糖、乳-N-三糖II和人乳低聚糖。

[0102] 根据第三方面,本申请提供了重组核酸分子,用于在细胞中增殖时表达唾液酸转移酶,当在细胞中表达时所述唾液酸转移酶为异源的唾液酸转移酶。所述重组核酸分子包含编码唾液酸转移酶的核苷酸序列,该唾液酸转移酶能够将唾液酸残基(例如N-乙酰神经氨酸残基)从供体底物转移到受体分子,其中所述受体分子选自乳糖、乳-N-三糖II和人乳低聚糖。

[0103] 在本文之前公开了与生产唾液酸化低聚糖的方法有关的编码唾液酸转移酶的核苷酸序列的优选实施方案,该唾液酸转移酶能够将唾液酸残基从供体底物转移到受体分子,其中所述受体分子选自乳糖、乳-N-三糖II和人乳低聚糖,例如优选的核苷酸序列。例如,唾液酸转移酶能够将N-乙酰神经氨酸残基从CMP-Neu5Ac转移到乳糖、乳-N-三糖II或人乳低聚糖。

[0104] 编码唾液酸转移酶的核苷酸序列可操作地连接到至少一种表达控制序列。因此,在一个额外的和/或替代的实施方案中,重组核酸分子包含至少一种表达控制序列,当所述重组核酸分子在细胞中增殖时,所述表达控制序列介导编码唾液酸转移酶的核苷酸序列的转录和/或翻译。

[0105] 根据第四方面,本申请提供了能够具有α-2,3-唾液酸转移酶活性和/或α-2,6-唾液酸转移酶活性的唾液酸转移酶,所述唾液酸转移酶活性将唾液酸残基(例如N-乙酰神经氨酸(Neu5Ac)残基)从供体底物(例如CMP-Neu5Ac)转移到受体分子,其中所述受体分子为乳糖、乳-N-三糖II或人乳低聚糖。

[0106] 在一个实施方案中,所述受体分子选自三糖、四糖和五糖。在一个额外的和/或替代的实施方案中,受体分子选自LST-a和LST-b。

[0107] 在一个额外的和/或替代的实施方案中,唾液酸转移酶选自:

[0108] I.包含SEQ ID NO:1至33中任一项所代表的氨基酸序列或由其组成的多肽;

[0109] II.包含与SEQ ID NO:1至33中任一项所代表的任一种氨基酸序列具有至少80%的序列相似性的氨基酸序列或由其组成的多肽;和

[0110] III.I和II中任一种多肽的片段。

[0111] 根据第五方面,本申请提供了本文之前记载的唾液酸转移酶用于生产唾液酸化低聚糖的用途,所述唾液酸转移酶能够将唾液酸残基从供体底物转移(例如将N-乙酰神经氨酸残基从CMP Neu5AC转移)到受体分子,其中所述受体分子为乳糖、乳-N-三糖II或人乳低聚糖。

[0112] 所述唾液酸化转移酶能够将唾液酸残基转移到受体分子中从而生产唾液酸化低聚糖,所述受体分子为人乳低聚糖。

[0113] 人乳低聚糖可是中性低聚糖或酸性低聚糖,即包含至少一个唾液酸残基的人乳低聚糖。

[0114] 如本文之前记载的,使用唾液酸转移酶产生的唾液酸化低聚糖可是人乳低聚糖或是在天然存在的人乳中没有发现的唾液酸化低聚糖。

[0115] 根据第六方面,本申请提供了一种用于通过体外生物催化生产唾液酸化低聚糖的方法,其中使用唾液酸转移酶,所述唾液酸转移酶能够将唾液酸残基从供体底物转移(例如将N-乙酰神经氨酸残基从CMP-Neu5Ac转移)到受体分子,其中所述受体分子为人乳低聚糖。

[0116] 所述方法包含以下步骤:

[0117] -在反应混合物中提供一种唾液酸转移酶,该酶能够将唾液酸残基(优选N-乙酰神经氨酸)从供体底物转移到受体分子、供体底物和受体分子;

[0118] -允许唾液酸转移酶将唾液酸残基从供体底物转移到受体分子,以获得唾液酸化低聚糖;并且

[0119] -从反应混合物中回收唾液酸化低聚糖。

[0120] 根据第七方面,本申请提供了通过根据第一方面的方法或根据第六方面的方法生产的唾液酸化低聚糖。

[0121] 在一个实施方案中,所述唾液酸化低聚糖为人乳低聚糖,优选四糖、五糖或六糖,更优选地选自LST-a、LST-b和DSLNT的唾液酸化低聚糖。

[0122] 根据第八方面,本申请提供了通过如本文之前记载的全细胞发酵方法或体外生物催化生产的唾液酸化低聚糖用于制备营养组合物的用途。所述营养组合物包含至少一种已经通过本文之前公开的方法生产的唾液酸化低聚糖。

[0123] 因此,根据第九方面,本申请提供了一种营养组合物,其包含至少一种已经通过本文之前公开的方法生产的唾液酸化低聚糖。优选地,所述至少一种唾液酸化低聚糖为3'-唾液酸乳糖、6'-唾液酸乳糖、LST-a、LST-b、LST-c或DSLNT。

[0124] 在一个额外的和/或替代的实施方案中,所述营养组合物还包含至少一种中性HMO,优选2'-FL。

[0125] 在一个额外的和/或替代的实施方案中,所述营养组合物包含3-SL、6-SL和2'-FL。

[0126] 在一个额外的实施方案中,所述营养组合物选自药用制剂、婴儿配方物和膳食补充剂。

[0127] 营养组合物可以液体形式或固体形式(包括但不限于粉末剂、颗粒剂(granule)、薄片和丸剂(pellet))存在。

[0128] 根据第十方面,本申请提供了一种婴儿配方物,其包含至少一种唾液酸化HMO。所述的唾液酸化HMO为选自通过本文之前记载的方法生产的唾液酸化低聚糖的HMO。

[0129] 在一个实施方案中,所述婴儿配方物中含有的至少一种唾液酸化HMO选自3-SL、6-SL、LST-a、LST-b、LST-c和DSLNT。

[0130] 在一个额外的和/或替代的实施方案中,婴儿配方物包含至少一种唾液酸化HMO和一种或多种中性HMO。

[0131] 在一个额外的和/或替代的实施方案中,婴儿配方物包含3-SL、6-SL和2'-FL。

[0132] 将用特定实施方案和附图来描述本发明,但本发明不仅限于此,而仅通过权利要求来限定。此外,说明书和权利要求中的术语第一、第二和类似术语用于区分相似的要素,而并不必然用于以时间、空间、排序或以任何其他方式描述顺序。应该理解,如此使用的术语在适当的情况下是可互换的,并且本文描述的本发明的实施方案能够以不同于本文描述

或举例说明的其他顺序操作。

[0133] 应当注意的是,权利要求中使用的术语“包括”不应被解释为仅限于其后列出的方法;它不排除其他要素或步骤。因此,它被解释为指明所述特征、整数、步骤或成分的存在,但不排除存在或添加一个或多个其他特征、整数、步骤或成分或其群组。因此,表述“包括装置A和B的设备”的范围不应限于仅由组件A和B组成的设备。这意味着,就本发明而言,该装置仅有的相关组件是A和B。

[0134] 在本说明书通篇中提及“一个(one)实施方案”或“一个(an)实施方案”是指在本发明的至少一个实施方案中包括就实施方案描述的特定的特性、结构或特征。因此,在本说明书通篇中各处出现的短语“在一个(one)实施方案中”或“在一个(an)实施方案中”不一定都是指同一实施方案,而是可能。此外,在一个或多个实施方案中,可以以任何适当的方式组合特定的特性、结构或特征,这对于本公开内容的技术人员来说是显而易见的。

[0135] 类似地,应当意识到,在本发明的示例性实施方案的描述中,为了简化本公开内容并有助于理解本发明的一个或多个方面,本发明的各种特征有时在单个实施方案、其图或描述中分组在一起。然而,这种公开方法不应被解释为反映了所要求保护的发明需要比在每个权利要求中明确陈述的特征更多的特征的意图。相反,正如下面的权利要求所反映的,本发明的方面小于单个前述公开实施方案的所有特征。因此,在具体的说明之后的权利要求被明确地纳入所述具体的说明中,每一项权利要求本身作为本发明的单独的实施方案。

[0136] 此外,虽然本文描述的一些实施方案包括其他实施方案中包括的一些特征但不包括其他特征,但不同实施方案的特征组合是指在本发明的范围内,并形成不同的实施方案,正如本领域技术人员所理解的那样。例如,在以下权利要求中,任何要求保护的实施方案都可以以任何组合使用。

[0137] 此外,本文将一些实施方案描述为可以由计算机系统的处理器或通过执行该功能的其他手段来实现的方法或方法要素的组合。因此,具有执行这种方法或方法要素的必要指令的处理器构成用于执行所述方法或方法要素的手段。此外,本文描述的装置实施方案的元件是为了实施本发明,用于执行由元件行使的功能的装置的实例。

[0138] 在本文提供的说明书和附图中,列出了许多具体的细节。然而,据了解,本发明的实施方案可以在没有这些具体细节的情况下实施。在其他情况下,众所周知的方法、结构和技术还没有被详细地展示出来,以避免混淆对本说明书的理解。

[0139] 现在将通过对本发明的几个实施方案的具体说明来描述本发明。显然,在不偏离本发明的真正主旨或技术教导的情况下,可以根据本领域技术人员的知识来设置本发明的其他实施方案,本发明仅受所附权利要求的术语限制。

[0140] 实施例

[0141] 实施例1:使得能够使用乳糖作为受体在体内筛选唾液酸转移酶的大肠杆菌Neu5Ac生产菌株的开发

[0142] 代谢工程化分别包括特定基因的诱变和缺失,以及异源基因的基因组整合。如Ellis等人(Proc.Natl.Acad.Sci.USA 98:6742-6746(2001))所记载的,通过使用错配寡核苷酸的诱变使基因lacZ和araA失活。

[0143] 根据Datsenko and Wanner(Proc.Natl.Acad.Sci.USA 97:6640-6645(2000))的方法进行基因组缺失。为了防止N-乙酰神经氨酸的胞内降解,编码N-乙酰葡糖胺-6-磷酸脱

乙酰酶(nagA)和葡糖胺-6-磷酸脱氨酶(nagB)的基因以及编码N-乙酰甘露糖胺激酶(nanK)、N-乙酰甘露糖胺-6-磷酸差向异构酶(nanE)、N-乙酰神经氨酸醛缩酶(nanA)和唾液酸通透酶(nanT)的整个N-乙酰神经氨酸分解代谢的基因簇,被从大肠杆菌BL21(DE3)菌株的基因组中缺失。基因wzxC-wcaJ也被缺失。WcaJ编码UDP-葡萄糖:十一异戊烯基磷酸葡萄糖-1-磷酸转移酶,其催化荚膜异多糖酸(colanic acid)合成的第一步(Stevenson et al.,J.Bac teriol.1996,178:4885-4893)。此外,还分别去除了编码L-岩藻糖异构酶和L-墨角藻糖激酶的基因fucl和fucK。

[0144] 通过转座进行异源基因的基因组整合。使用EZ-Tn5$^{TM}$转座酶(Epic entre,USA)整合线性DNA片段或将水手转座酶Himar1的超活性C9-突变体用于转座(Lampe et al.,Proc.Natl.Acad.Sci.1999,USA 96:11428-1 1433)。为了生产EZ-Tn5转座体,用引物1119和1120(所有使用的引物列于下表3)扩增目的基因和侧翼为FRT位点的抗生素抗性标记;得到的PCR产物在两个位点上均携带用于EZ-Tn5转座酶的19-b p嵌合端识别位点。对于使用Himar1转座酶进行的整合,将目的表达构建体(操纵子)以及侧翼为FRT位点的抗生素抗性标记一起类似地克隆到pEcomar载体中。在阿拉伯糖诱导型启动子$P_{araB}$的控制下,pEcomar载体编码水手转座酶Himar1的超活性C9-突变体。使用EZ-Tn5转座酶整合表达片段<$P_{tet}$-lacY-FRT-aadA-FRT>(SEQ ID NO:67)。在成功整合来自大肠杆菌K12 TG1的乳糖输入蛋白LacY(登录号ABN72583)的基因后,通过在质粒pCP20上编码的FLP重组酶从链霉素抗性克隆中消除抗性基因(Datsenko and Wanner,Proc.Natl.Acad.Sci.2000,USA 97:6640-6645),得到菌株#534。此外,大肠杆菌W的csc-基因簇(登录号CP002185.1)被插入到基因组中,所述基因簇包含能够使该菌株在作为唯一碳源的蔗糖上生长的蔗糖通透酶、果糖激酶、蔗糖水解酶和转录抑制因子的基因(分别为基因cscB、cscK、cscA和cscR)。该csc-基因簇通过使用质粒pEcomar-cscABKR进行的转座整合到大肠杆菌BL21(DE3)菌株的基因组中。为提高UDP-N-乙酰葡糖胺的从头合成,对编码L-谷氨酰胺:D-果糖-6-磷酸氨基转移酶的基因(glmS)、编码大肠杆菌K-12次代菌株MG1655的磷酸葡糖胺变位酶的基因(glmM)和编码大肠杆菌K-12次代菌株MG1655的N-乙酰葡糖胺-1-磷酸尿苷酰转移酶/葡糖胺-1-磷酸乙酰转移酶的基因(glmU)(登录号分别为NP_418185、NP_417643、NP_418186)进行密码子优化并通过基因合成获得。将操纵子glmUM克隆到组成型四环素启动子$P_{tet}$的控制下,同时将glmS克隆到组成型$P_{T5}$启动子的控制下。插入来自pEcomar-glmUM-glmS的转座子盒<$P_{tet}$-glmUM-$P_{T5}$-glmS-FRT-dhfr-FRT>(SEQ ID NO:68),其侧翼为由类水手元件Himar1转座酶特异性识别的反向末端重复。总之,所记载的基因组修饰导致得到大肠杆菌BL21(DE3)菌株#942,其代表了菌株开发的基础。表1、表2和表3分别包含所有菌株、用于克隆的寡核苷酸以及本研究中使用的常见质粒。

[0145] 通过表达盒<$P_{tet}$-glmSm-gna1-FRT-aacC1-FRT>(SEQ ID NO:69)、<$P_{tet}$-slr1975-FRT-cat-FRT>(SEQ ID NO:70)、<$P_{tet}$-neuBC-FRT-kan-FRT>(SEQ ID NO:71)和<$P_{tet}$-ppsA-FRT-aad1-FRT>(SEQ ID NO:72)的基因组整合,对菌株#942进行修饰,以用于生产唾液酸。所有基因均进行密码子优化以在大肠杆菌中表达,并通过GenScript公司以合成方法制备。GlmSm代表了一种诱变形式的GlmS,从而消除了葡糖胺-6-磷酸的反馈抑制作用。基因gna1编码来自酿酒酵母的葡糖胺-6-磷酸乙酰转移酶。使用引物glmSm/gna1_1-8,将基因亚克隆为组成型启动子$P_{tet}$后面的操纵子,并与侧翼为FRT-位点的庆大霉素抗性基因融合。类似

地,使用引物neuBC_1-6,将均来源于空肠弯曲杆菌(Campylobacter jejuni)的编码唾液酸合酶的基因neuB(登录号AF305571)和编码UDP-N-乙酰葡糖胺-2-差向异构酶的基因neuC(登录号AF305571)亚克隆为组成型启动子$P_{tet}$后面的操纵子,并与侧翼为FRT位点的卡那霉素抗性基因融合。还使用引物slr_1-4将基因slr1975(登录号BAL35720)克隆到组成型启动子$P_{tet}$后面,并与侧翼为FRT位点的氯霉素抗性基因融合,基因slr1975编码来自集胞藻属种(Synechocystis sp.)PCC6803的N-乙酰葡糖胺2-差向异构酶。使用引物ppsA_1-4,将编码大肠杆菌BL21(DE3)的磷酸烯醇丙酮酸合酶的基因ppsA(登录号ACT43527)类似地克隆以进行组成型表达,并与侧翼为FRT位点的链霉素抗性基因融合。基因组整合最终导致得到Neu5Ac生产菌株#1363,其用于筛选唾液酸转移酶1至26。

[0146]

| 菌株 | 基因型 | 参考文献 |
|---|---|---|
| *E. coli* BL21(DE3) | F– *ompT hsd*SB(rB–, mB–) *gal dcm* (DE3) | Merck KGaA, Darmstadt, Germany |
| *E. coli* BL21(DE3) #287 | *E. coli* BL21(DE3) *ΔlacZ* | **本研究** |
| *E. coli* BL21(DE3) #534 | *E. coli* BL21(DE3) *ΔlacZ Δara ΔwcaJ ΔfuclK ΔnagAB* **，携带 *galETKM*、*lacy* 的基因组整合** | **本研究** |
| *E. coli* BL21(DE3) #942 | *E. coli* BL21(DE3) *ΔlacZ Δara ΔwcaJ ΔfuclK ΔnagAB ΔnanKETA::zeo* **，携带 *galETKM*、*lacy*、*cscBKAR*、*glmUM-glmS-dhfr* 的基因组整合** | **本研究** |
| *E. coli* BL21(DE3) #1363 | *E. coli* BL21(DE3) *ΔlacZ Δara ΔwcaJ ΔfuclK ΔnagAB ΔnanKETA::zeo* **，携带 *galETKM*、*lacy*、*cscBKAR*、*glmUM-glmS-dhfr*、*glmSm-gna1-aacC1*、*slr1975-cat*、*neuBC-kan*、*ppsA-aad1* 的基因组整合** | **本研究** |
| *E. coli* BL21(DE3) #1730 | *E. coli* BL21(DE3) *ΔlacZ Δara ΔwcaJ ΔfuclK ΔnagAB ΔnanKETA::zeo* **，携带 *galETKM*、*lacy*、*cscBKAR*、*glmUM-glmS-dhfr*、*neuA-nanT-kan* 的基因组整合** | **本研究** |
| *E. coli* BL21(DE3) #2130 | *E. coli* BL21(DE3) *ΔlacZ Δara ΔwcaJ ΔfuclK ΔnagAB ΔnanKETA* **，携带 *galETKM*、*lacY*、*lgtA-galT-kan*、*glmUM-glmS-dhfr*、*wbdO-galE-cat* 、*neuA-nanT-aacC1* 的基因组整合** | **本研究** |

[0147] 表1:所使用的细菌菌株列表。

[0148]

| 引物 | 序列（5’-3’） | SEQ ID NO: |
|---|---|---|
| 1119 | P-CTGTCTCTTATACACATCTCCTGAAATTGGCCA GATGATTAATTCCTAATTTTTGTTG | 74 |
| 1120 | P-CTGTCTCTTATACACATCTCAGCATTACACGTC TTGAGCGATTGTGTAGG | 75 |
| slr_1 | CCTGACGACGGTGAGCGATCATTTGTATATCTCC TTCTTAAAGTTAAACAAAATTATTTC | 76 |
| slr_2 | AACCCTGCAACTGCCGGTCTCTTAAAATAACTAG CATAACCCCTTGGGGCCTCTAAACG | 77 |
| slr_3 | AACTTTAAGAAGGAGATATACAAATGATCGCTCA CCGTCGTCAGGAACTGGCTCAACAG | 78 |
| slr_4 | AGGCCCCAAGGGGTTATGCTAGTTATTTTAAGAG ACCGGCAGTTGCAGGGTTTCGGC | 79 |
| glmSm/gna1_1 | GCCCCAAGGGGTTATGCTAGTTATTTTATTCCAC GGTCACGGATTTCGCCAGGTTACGCGGC | 80 |
| glmSm/gna1_2 | AGCACCAACGATACCGCACATTTGTATATCTCCT TCTTAAAGTTAAACAAAATTATTTCTAG | 81 |
| glmSm/gna1_3 | GCGAAATCCGTGACCGTGGAATAAAATAACTAGC ATAACCCCTTGGGGCCTCTAAACGGGTC | 82 |
| glmSm/gna1_4 | CTTTAAGAAGGAGATATACAAATGTGCGGTATCG TTGGTGCTATCGCACAGCGTGATG | 83 |
| glmSm/gna1_5 | CGTGGAAATGCAAATTAGAAAATAGAATAACTAG CATAACCCCTTGGGGCCTCTAAACGG | 84 |
| glmSm/gna1_6 | TATAAAATCCATCGGGTAAGCTCATGGTTCTATC TCCTTCGTTATTCCACGGTCACGGATTTCG | 85 |
| glmSm/gna1_7 | ATCCGTGACCGTGGAATAACGAAGGAGATAGAA CCATGAGCTTACCCGATGGATTTTATATAAGG | 86 |
| glmSm/gna1_8 | CCGTTTAGAGGCCCCAAGGGGTTATGCTAGTTAT TCTATTTTCTAATTTGCATTTCCACG | 87 |
| ppsA_1 | CTGGTTAAGCCTGGCTGAACTGAAGAAATAAAAT AACTAGCATAACCCCTTGGGGCCTC | 88 |
| ppsA_2 | TAGAGGCCCCAAGGGGTTATGCTAGTTATTTTAT TTCTTCAGTTCAGCCAGGCTTAACC | 89 |
| ppsA_3 | TTTGTTTGGCGTCGAGAAGGAGATAGAACCATGT CCAACAATGGCTCGTCACCGCTGGTG | 90 |
| ppsA_4 | AAGCACCAGCGGTGACGAGCCATTGTTGGACAT GGTTCTATCTCCTTCTCGACGCCAAAC | 91 |
| neuBC_1 | CTACCCAGAAAGTGTTCAAAGATATTAAATAAAAT AACTAGCATAACCCCTTGGGGCCTC | 92 |
| neuBC_2 | TTTAGAGGCCCCAAGGGGTTATGCTAGTTATTTT ATTTAATATCTTTGAACACTTTCTGGG | 93 |
| neuBC_3 | GATGATGATGTTCTGGATTTTGATTTCTTTCATTT GTATATCTCCTTCTTAAAGTTAAAC | 94 |

[0149]

| 引物 | 序列（5’ - 3’） | SEQ ID NO: |
|---|---|---|
| neuBC_4 | TTTGTTTAACTTTAAGAAGGAGATATACAAATGAAAGAAATCAAAATCCAGAACATCATC | 95 |
| neuBC_5 | AGCTGTCTTATGAAGATTTCGCCTAATCGAAGGAGATACAACCATGAAGAAAATTCTGTTTATCACCGGC | 96 |
| neuBC_6 | TGCCGGTGATAAACAGAATTTTCTTCATGGTTGTATCTCCTTCGATTAGGCGAAATCTTCATAAGACAGC | 97 |
| neuA/nanT_1 | TAATTTTGTTTAACTTTAAGAAGGAGATATACATGAGCCTGGCCATTATCCCGGCACGTG | 98 |
| neuA/nanT_2 | TGCCGGGATAATGGCCAGGCTCATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTC | 99 |
| neuA/nanT_3 | AAAATAAGAGCTCGAGTCGAAGGAGATAGAACCATGAGTACTACAACCCAGAATATCCCG | 100 |
| neuA/nanT_4 | TAGTACTCATGGTTCTATCTCCTTCGACTCGAGCTCTTATTTTTTCCAGATCTGTTCCAC | 101 |
| neuA/nanT_5 | AAAACGATTTAGTCAAAACCAAAAGTTAATAAATCGATACTAGCATAACCCCTTGGGGCC | 102 |
| neuA/nanT_6 | AAGGGGTTATGCTAGTATCGATTTATTAACTTTTGGTTTTGACTAAATCGTTTTTG | 103 |
| 6192 nst-neuA attB1 | GGGGACAAGTTTGTACAAAAAAGCAGGCTAGAAGGAGGTATACAAATGGGCCTGAAAAAAGCCTGCCTGACCG | 104 |
| 6193 cst-neuA attB1 | GGGGACAAGTTTGTACAAAAAAGCAGGCTAGAAGGAGATATACAAATGACCCGCACCCGTATGGAAAACGAACTG | 105 |
| 6194 siaT-neuA attB2 | GGGGACCACTTTGTACAAGAAAGCTGGGTTTATTTTTTCCAGATCTGTTCCACTTTTTTCAG | 106 |
| 6559 siaT3 for | AAAAAGCAGGCTAGAAGGAGGTATACAAATGGGCAAAAAAGTGATTATTGCGGGCAACGGCCCGAGCC | 107 |
| 6560 siaT3 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTAGTTGATGTTTTTGCTGAATTTGCCATACGCTTCGC | 108 |
| 6561 neuA (siaT3) for | TGGCAAATTCAGCAAAAACATCAACTAAACCTCGAAGGAGGTACAATTATGAGCCTGGCCATTATCCC | 109 |
| 6562 pDest (siaT3) rev | TGCCCGCAATAATCACTTTTTTGCCCATTTGTATACCTCCTTCTAGCCTGCTTTTTTGTACAAACTTG | 110 |
| 6563 siaT4 for | AAAAGCAGGCTAGAAGGAGGTATACAAATGAATAAGAAACCGCTGATTATTGCTGGCAACGGGCC | 111 |
| 6564 siaT4 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTATCTCTTCAGGAATGCTTTAATGATTGACTTTAGCGCC | 112 |
| 6565 neuA (siaT4) for | ATCATTAAAGCATTCCTGAAGAGATAAACCTCGAAGGAGGTACAATTATGAGCCTGGCCATTATC | 113 |

[0150]

| 引物 | 序列（5’-3’） | SEQ ID NO: |
|---|---|---|
| 6566 pDest (siaT4) rev | AGCAATAATCAGCGGTTTCTTATTCATTTGTATAC CTCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 114 |
| 6567 siaT5 for | AAAAGCAGGCTAGAAGGAGGTATACAAATGGGG ACCATTAAAAAGCCCTTAATCATAGCAGGAAATG | 115 |
| 6568 siaT5 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTATGC AGCTCCCCAACGGAAACTAACTTTTAATGTTGGG | 116 |
| 6569 neuA (siaT5) for | TAGTTTCCGTTGGGGAGCTGCATAAACCTCGAA GGAGGTACAATTATGAGCCTGGCCATTATCCCG | 117 |
| 6570 pDest (siaT5) rev | ATTAAGGGCTTTTTAATGGTCCCCATTTGTATAC CTCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 118 |
| 6748 siaT6 for | AAAAGCAGGCTAGAAGGAGGTATACAAATGAGT GAAGAAAACACCCAGTCCATTATTAAAAACGAC | 119 |
| 6749 siaT6 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTCAGAC AGCAATACAGACACCCGTTTCGCAATTCGGCAG | 120 |
| 6750 neuA (siaT6) for | AAACGGGTGTCTGTATTGCTGTCTGAACCTCGAA GGAGGTACAATTATGAGCCTGGCCATTATCCCG | 121 |
| 6751 pDest (siaT6) rev | ATGGACTGGGTGTTTTCTTCACTCATTTGTATAC CTCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 122 |
| 6752 siaT7 for | AAAAGCAGGCTAGAAGGAGGTATACAAATGACCA TTTACCTGGACCCGGCGTCTCTGCCGACCC | 123 |
| 6753 siaT7rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTACAG TTGTTTCAGAGAATCCCAGAAGATAATTTGGC | 124 |
| 6754 neuA (siaT7) for | TTCTGGGATTCTCTGAAACAACTGTAAACCTCGA AGGAGGTACAATTATGAGCCTGGCCATTATCCC | 125 |
| 6755 pDest (siaT7) rev | ACGCCGGGTCCAGGTAAATGGTCATTTGTATACC TCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 126 |
| 6483 siaT8 for | AAAGCAGGCTAGAAGGAGGTATACAAATGGGCT GTAATAGCGACTCCAACCACAACAACTCCGACG | 127 |
| 6484 siaT8 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTATTG CAGGTCCGAGATCAGTTTCACATCATTACGG | 128 |
| 6485 neuA (siaT8) for | TGAAACTGATCTCGGACCTGCAATAAACCTCGAA GGAGGTACAATTATGAGCCTGGCCATTATCCC | 129 |
| 6486 pDest (siaT8) rev | TGGTTGGAGTCGCTATTACAGCCCATTTGTATAC CTCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 130 |
| 6487 siaT9 for | AAAGCAGGCTAGAAGGAGGTATACAAATGAACAA CGACAACTCCACGACCACCAACAATAACGC | 131 |
| 6488 siaT9 rev | CTCATAATTGTACCTCCTTCGAGGTTTAAATGTCA GAGATCAGTTTAATATTATCGCGGTTAATCAG | 132 |
| 6489 neuA (siaT9) for | ATATTAAACTGATCTCTGACATTTAAACCTCGAAG GAGGTACAATTATGAGCCTGGCCATTATCCCG | 133 |
| 6490 pDest (siaT9) rev | TGGTCGTGGAGTTGTCGTTGTTCATTTGTATACC TCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 134 |
| 6491 siaT10 for | AAAGCAGGCTAGAAGGAGGTATACAAATGAAAAC GATTACCCTGTATCTGGACCCGGCGTCCCTGCC | 135 |

[0151]

| 引物 | 序列（5’-3’） | SEQ ID NO: |
|---|---|---|
| 6492 siaT10 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTACAG CTGTTTCAGGCTGTCCCAAAAGATCACTTGCG | 136 |
| 6493 neuA (siaT10) for | TTTGGGACAGCCTGAAACAGCTGTAAACCTCGAA GGAGGTACAATTATGAGCCTGGCCATTATCCCG | 137 |
| 6494 pDest (siaT10) rev | TCCAGATACAGGGTAATCGTTTTCATTTGTATAC CTCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 138 |
| 6495 siaT11 for | AAAGCAGGCTAGAAGGAGGTATACAAATGAAAAA GATCCTGACCGTCCTGAGCATCTTTATCCTGAGC | 139 |
| 6496 siaT11 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTAGTC CAGCATCGTACCGAAGTCATCCGGTTTGGTGTG | 140 |
| 6497 neuA (siaT11) for | ATGACTTCGGTACGATGCTGGACTAAACCTCGAA GGAGGTACAATTATGAGCCTGGCCATTATCCCG | 141 |
| 6498 pDest (siaT11) rev | TGCTCAGGACGGTCAGGATCTTTTTCATTTGTAT ACCTCCTTCTAGCCTGCTTTTTTGTACAAACTTGT | 142 |
| 6503 siaT13 for | AAAGCAGGCTAGAAGGAGGTATACAAATGACGA ATCGCAAAATCTATGTCTGCCACACCCTGTACC | 143 |
| 6504 siaT13 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTATTT AATGTCTTTCAGATCAACCAGCGTAATTTTCTTGT | 144 |
| 6505 neuA (siaT13) for | TGGTTGATCTGAAAGACATTAAATAAACCTCGAA GGAGGTACAATTATGAGCCTGGCCATTATCCC | 145 |
| 6506 pDest (siaT13) rev | AGACATAGATTTTGCGATTCGTCATTTGTATACCT CCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 146 |
| 6507 siaT14 for | AAAGCAGGCTAGAAGGAGGTATACAAATGTTCCG TGAAGACAATATGAACCTGATTATCTGCTGTACG | 147 |
| 6508 siaT14 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTAGAT GTCGATAACTTTGATACCGAAATCTTTCAGG | 148 |
| 6509 neuA (siaT14) for | TCGGTATCAAAGTTATCGACATCTAAACCTCGAA GGAGGTACAATTATGAGCCTGGCCATTATCCCG | 149 |
| 6510 pDest (siaT14) rev | AGGTTCATATTGTCTTCACGGAACATTTGTATAC CTCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 150 |
| 6511 siaT15 for | AAAGCAGGCTAGAAGGAGGTATACAAATGAAAGA AATCGCCATCATCTCCAACCAACGCATGTTCTTC | 151 |
| 6512 siaT15 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTAGTC AAAGAAATCCAGCAGTTTCGGATGCACCGCGGT | 152 |
| 6513 neuA (siaT15) for | TCCGAAACTGCTGGATTTCTTTGACTAAACCTCG AAGGAGGTACAATTATGAGCCTGGCCATTATCCC | 153 |
| 6514 pDest (siaT15) rev | TTGGAGATGATGGCGATTTCTTTCATTTGTATAC CTCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 154 |
| 6515 siaT16 for | AAAGCAGGCTAGAAGGAGGTATACAAATGCTGAT TCAACAGAACCTGGAAATCTACCTGGACTACGC | 155 |
| 6516 siaT16 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTAATT GTGAATGGTGCACATAAACGCCTGATCTTCGTTG | 156 |
| 6517 neuA (siaT16) for | AGGCGTTTATGTGCACCATTCACAATTAAACCTC GAAGGAGGTACAATTATGAGCCTGGCCATTATCC | 157 |

[0152]

| 引物 | 序列（5’-3’） | SEQ ID NO: |
|---|---|---|
| 6518 pDest (siaT16) rev | ATTTCCAGGTTCTGTTGAATCAGCATTTGTATAC CTCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 158 |
| 6519 siaT17 for | AAAGCAGGCTAGAAGGAGGTATACAAATGGGCT GTAACTCCGATAGCAAACACAATAACAGTGATGG | 159 |
| 6520 siaT17 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTATTG CAGGTCACTAATCAGTTTCACATCATTGCGG | 160 |
| 6521 neuA (siaT17) for | TGAAACTGATTAGTGACCTGCAATAAACCTCGAA GGAGGTACAATTATGAGCCTGGCCATTATCCCG | 161 |
| 6522 pDest (siaT17) rev | TGTTTGCTATCGGAGTTACAGCCCATTTGTATAC CTCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 162 |
| 6523 siaT18 for | AAAGCAGGCTAGAAGGAGGTATACAAATGTGTAA CGATAATCAAAATACGGTCGATGTTGTTGTGAGC | 163 |
| 6524 siaT18 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTAATA CTGAGCAATACAAACACCCGAGGAACAATCCGG | 164 |
| 6525 neuA (siaT18) for | TCGGGTGTTTGTATTGCTCAGTATTAAACCTCGA AGGAGGTACAATTATGAGCCTGGCCATTATCCC | 165 |
| 6526 pDest (siaT18) rev | ACCGTATTTTGATTATCGTTACACATTTGTATACC TCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 166 |
| 6807 siaT19 for | AAAAGCAGGCTAGAAGGAGGTATACAAATGAAC GATAATCAAAATACGGTGGACGTGGTGGTCTC | 167 |
| 6808 siaT19 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTAGCA CCAGAACAGCACATCTTTTTCTTTCACAATGCC | 168 |
| 6809 neuA (siaT19) for | AAAAAGATGTGCTGTTCTGGTGCTAAACCTCGAA GGAGGTACAATTATGAGCCTGGCCATTATCCCG | 169 |
| 6810 pDest (siaT19) rev | TCCACCGTATTTTGATTATCGTTCATTTGTATACC TCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 170 |
| 6811 siaT20 for | AAAAGCAGGCTAGAAGGAGGTATACAAATGCAAA ACGTCATTATCGCTGGTAACGGTCCGAGCCTGC | 171 |
| 6812 siaT20 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTATTT CTTTTTGTATTCTTTCTTCAGTTTTTTGATTTCG | 172 |
| 6813 neuA (siaT20) for | TGAAGAAAGAATACAAAAAGAAATAAACCTCGAA GGAGGTACAATTATGAGCCTGGCCATTATCCCG | 173 |
| 6814 pDest (siaT20) rev | TTACCAGCGATAATGACGTTTTGCATTTGTATAC CTCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 174 |
| 6815 siaT21 for | AAAAGCAGGCTAGAAGGAGGTATACAAATGGATT CTTCGCCGGAAAACACCAGCTCTACGCTGG | 175 |
| 6816 siaT21 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTATTT GATGTCCGTCGTAAAGCGCACTTTTTCGTCCG | 176 |
| 6817 neuA (siaT21) for | TGCGCTTTACGACGGACATCAAATAAACCTCGAA GGAGGTACAATTATGAGCCTGGCCATTATCCCG | 177 |
| 6818 pDest (siaT21) rev | TGGTGTTTTCCGGCGAAGAATCCATTTGTATACC TCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 178 |
| 6652 siaT22 for | AAAAGCAGGCTAGAAGGAGGTATACAAATGAAGA AAGTCTACTTCTGCCATACGGTCTACCATCTGC | 179 |

[0153]

| 引物 | 序列（5’-3’） | SEQ ID NO: |
|---|---|---|
| 6653 siaT22 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTAACT ATTTGCTTTCATTTGTTTCAGGGTGATTTTC | 180 |
| 6654 neuA (siaT22) for | TGAAACAAATGAAAGCAAATAGTTAAACCTCGAA GGAGGTACAATTATGAGCCTGGCCATTATCCCG | 181 |
| 6655 pDest (siaT22) rev | TATGGCAGAAGTAGACTTTCTTCATTTGTATACCT CCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 182 |
| 6819 siaT24 for | AAAGCAGGCTAGAAGGAGGTATACAAATGCGTAA AATCATCACCTTCTTCAGCCTGTTCTTCTCG | 183 |
| 6820 siaT24 rev | AGGCTCATAATTGTACCTCCTTCGAGGTTTAAAA GTTAATCGGGTTCGGCATTTCTTCAAAGAAAATC | 184 |
| 6821 neuA (siaT24) for | AAATGCCGAACCCGATTAACTTTTAAACCTCGAA GGAGGTACAATTATGAGCCTGGCCATTATCCCG | 185 |
| 6822 pDest (siaT24) rev | TGAAGAAGGTGATGATTTTACGCATTTGTATACC TCCTTCTAGCCTGCTTTTTTGTACAAACTTGTG | 186 |

[0154] 表2:用于PCR的寡核苷酸列表

[0155] 实施例2:使得能够使用乳糖为受体但需要外源添加唾液酸在体内筛选唾液酸转移酶的大肠杆菌菌株的开发

[0156] 用大肠杆菌BL21(DE3)菌株#942建立用于编码唾液酸转移酶27至100的质粒的筛选菌株。因此,为了使得能够进行唾液酸的摄取和核苷酸活化,基因nanT和neuA分别被整合。编码大肠杆菌Neu5Ac主要易化子超家族转运蛋白的nanT基因(登录号B21_03035)是从大肠杆菌BL21(DE3)的基因组DNA扩增,以及对来自空肠弯曲杆菌的neuA基因(登录号AF305571)进行密码子优化,并通过合成得到。这些基因被克隆为在组成型四环素启动子$P_{tet}$的控制下的操纵子,并用EZ-Tn5转座酶对所得到的表达片段<$P_{tet}$-neuA-nanT-lox66-kan-lox72>(SEQ ID NO:73)进行整合,得到筛选菌株#1730。

[0157]

| 质粒 | 相关基因型 | 参考文献 |
|---|---|---|
| pCP20 | rep, *FLP*, *bla*, *cat* | Datsenko and Wanner, 2002 |
| pEcomar-*cscABKR* | 大肠杆菌 *W* 的 *cscB*、*cscK*、*cscA* 和 *cscR* 整合到载体 pEcomar; *bla* | 本研究 |
| pEcomar-*glmUM-glmS* | 大肠杆菌 K12 的 *glmU*、*glmM* 和 *glmS* 整合到载体 pEcomar; *dhfr*, *bla* | 本研究 |
| pEcomar-*lgtA-galT* | 脑膜炎奈瑟氏菌的 *lgtA* 和大肠杆菌 K12 的 *galT* 整合到载体 pEcomar; *kan*, *bla* | 本研究 |
| pEcomar-*wbdO-galE* | 肠道沙门氏菌萨拉梅亚种 Greenside 血清型（*Salmonella enterica* subsp. *salamae* serovar Greenside）的 *wbdO* 和大肠杆菌 K12 的 *galE* 整合到载体 pEcomar; *cat*, *bla* | 本研究 |
| pDEST14 | *bla*, *cat*, *ccdB* | Thermo Fisher Scientific, Waltham, Massachusetts, USA |

[0158] 表3:使用的常见质粒列表

[0159] 实施例3:产生编码唾液酸转移酶的质粒种质库(collection)

[0160] 从文献和公共数据库中接收到表征的或推定的唾液酸转移酶的基因序列。由于唾液酸转移酶通常被描述为在其信号肽缺失时表现出更高的活性,本发明人用在线预测工具SignalP(Petersen et al.,Nature Method s,2011Sep 29;8(10):785-6)分析了对应的蛋白质序列。基因是由GenS cript公司通过合成方法合成,其或者为注释的全长形式,或当信号肽被预测时,作为缺乏N端信号肽的截短变体(表4)。

[0161] 通过使用基因特异性引物(表2)的SLIC将唾液酸转移酶1至26分别作为操纵子与neuA一起亚克隆到pDEST14中,产生常用类型的质粒:pDEST14-siaT-neuA。其余的唾液酸转移酶27至100是通过GenScript公司使用限制性位点NdeI和BamHI直接亚克隆到质粒pET11a中。这两个表达系统都允许IPTG-诱导型基因表达(图2)。在体内活性筛选中,质粒被转化到菌株#1363或#1730,而大肠杆菌BL21(DE3)野生型或其lacZ-缺失变体(菌株#287)用于体外试验。

[0162]

| 质粒 | 唾液酸转移酶基因的起源 | 唾液酸转移酶基因的登录号 | 克隆为全长(FL)基因或不含信号肽(△) |
|---|---|---|---|
| pDEST14-siaT1-neuA | 脑膜炎奈瑟氏菌 | U60660 | FL (SEQ ID NO: 5) |
| pDEST14-siaT2-neuA | 空肠弯曲杆菌菌株 OH4384 | AF130466 | FL (SEQ ID NO: 10) |
| pDEST14-siaT3-neuA | 空肠弯曲杆菌菌株 OH4384 | AX934425 | FL (SEQ ID NO: 12) |
| pDEST14-siaT4-neuA | 蓖麻毒素螺杆菌（*Helicobacter acinonychis*） | NC_008229 | FL (SEQ ID NO: 11) |
| pDEST14-siaT5-neuA | 蓖麻毒素螺杆菌 | NC_008229 | FL (SEQ ID NO: 33) |
| pDEST14-siaT6-neuA | 发光杆菌属种(*Photobacterium* sp.) JT-ISH-224 | BAF92026 | Δ17 (SEQ ID NO: 29) |
| pDEST14-siaT7-neuA | 达可马巴斯德氏菌(*Pasteurella dagmatis* )菌株 DSM 22969 | AFY98851 | FL (SEQ ID NO: 7) |
| pDEST14-siaT8-neuA | 发光杆菌属种 JT-ISH-224 | BAF92025 | Δ20 (SEQ ID NO: 3) |
| pDEST14-siaT9-neuA | 弧菌属种(*Vibrio* sp.) JT-FAJ-16 | BAF91160 | Δ22 (SEQ ID NO: 2) |
| pDEST14-siaT10-neuA | 多杀性巴氏杆菌(*Pasteurella multocida*) PM70 | AAK02272 | Δ25 (SEQ ID NO: 4) |
| pDEST14-siaT11-neuA | 美人鱼发光杆菌(*Photobacterium damselae*) JT0160 | BAA25316 | FL (SEQ ID NO: 31) |
| pDEST14-siaT13-neuA | 无乳链球菌(*Streptococcus agalactiae* ) | AB050723 | FL (SEQ ID NO: 17) |
| pDEST14-siaT14-neuA | 睡眠嗜血杆菌(*Haemophilus somnus*)-2336 | ACA31578 | FL (SEQ ID NO: 26) |
| pDEST14-siaT15-neuA | 杜克雷嗜血杆菌(*Haemophilus ducreyi*) 35000HP | AF101047 | FL (SEQ ID NO: 14) |
| pDEST14-siaT16-neuA | 杜克雷嗜血杆菌 35000HP | AAP95068 | FL (SEQ ID NO: 27) |
| pDEST14-siaT17-neuA | 明亮发光杆菌(*Photobacterium phosphoreum*)JI-ISH-467 | BAF63530 | Δ20 (SEQ ID NO: 8) |
| pDEST14-siaT18-neuA | 鳎鱼发光杆菌(*Photobacterium leiognathi*) JT-SHIZ-119 | AB500947 | Δ15 (SEQ ID NO: 28) |
| pDEST14-siaT19-neuA | 鳎鱼发光杆菌 JT-SHIZ-145 | BAF91416 | Δ15 (SEQ ID NO: 30) |
| pDEST14-siaT20-neuA | 结肠弯曲杆菌(*Campylobacter coli*) | YP_008473374 | FL (SEQ ID NO: 1) |
| pDEST14-siaT21-neuA | 哈氏弧菌(*Vibrio harveyi*) | WP_017817635 | Δ24 (SEQ ID NO: 21) |

[0163]

| 质粒 | 唾液酸转移酶基因的起源 | 唾液酸转移酶基因的登录号 | 克隆为全长(FL)基因或不含信号肽(△) |
|---|---|---|---|
| pDEST14-siaT22-neuA | 肠链球菌(*Streptococcus entericus*) | WP_018369230 | FL (SEQ ID NO: 13) |
| pDEST14-siaT24-neuA | 副鸡禽杆菌(*Avibacterium paragallinarum*) | WP_021724759 | FL (SEQ ID NO: 9) |
| pET11a-siaT30 | 副溶血嗜血杆菌(*Haemophilus parahaemolyticus*)HK385 | EIJ71207 | FL (SEQ ID NO: 19) |
| pET11a-siaT31 | *Alistipes* sp. CAG:268 | CDC95697 | Δ17 (SEQ ID NO: 22) |
| pET11a-siaT32 | *Alistipes* sp. AL-1 | WP_032134786 | FL (SEQ ID NO: 15) |
| pET11a-siaT34 | 多杀性巴氏杆菌 PM70 | NC_002663 | FL (SEQ ID NO: 6) |
| pET11a-siaT37 | 空肠弯曲杆菌菌株 81-176 | AAL09368 | FL (SEQ ID NO: 16) |
| pET11a-siaT39 | *Alistipes shahii* WAL 8301 | YP_007816735 | Δ21 (SEQ ID NO: 23) |
| pET11a-siaT41 | 猪放线杆菌(*Actinobacillus suis*) ATCC 33415 | AIJ32009 | FL (SEQ ID NO: 24) |
| pET11a-siaT42 | 荚膜放线杆菌(*Actinobacillus capsulatus*) DSM 19761 | WP_018652686 | FL (SEQ ID NO: 25) |
| pET11a-siaT43 | *Bibersteinia trehalosi USDA-ARS-USMARC-189* | AHG84654 | FL (SEQ ID NO: 18) |
| pET11a-siaT51 | 美人鱼发光杆菌美人鱼亚种 CIP 102761 | EEZ40509 | FL (SEQ ID NO: 32) |
| pET11a-siaT73 | 睡眠嗜血杆菌 2336 | ACA31170 | FL (SEQ ID NO: 20) |

[0164] 表4:编码唾液酸转移酶的质粒列表。将唾液酸转移酶1至11、13至21和24作为操纵子与空肠弯曲杆菌的neuA(登录号AY102622)一起克隆。通过位点NdeI和BamHI克隆唾液酸转移酶30至32、34、37、39、41、42、51和73。将唾液酸转移酶基因克隆为全长构建体(FL)或无预测的信号肽(△)。△后面的数字表示从对应序列中缺失的N端氨基酸。

[0165] 实施例4:以乳糖为受体底物的α-2,3-和α-2,6-唾液酸转移酶的鉴定和表征

[0166] 携带编码100种唾液酸转移酶的质粒的大肠杆菌BL21(DE3)#1363和#1730在装有补充了2%(wt/vol)葡萄糖、氨苄西林100μg $ml^{-1}$、卡那霉素15μg $ml^{-1}$和博莱霉素40μg $ml^{-1}$的20ml无机盐培养基(Sam ain et al.,J.Biotech.1999,72:33-47)的100ml摇瓶中在30℃下生长。当培养物达到$OD_{600}$为0.1至0.3时,通过添加0.3mM IPTG诱导基因表达。孵育1小时后,添加1.5mM的乳糖到#1363培养物中,而添加1.5mM的乳糖和1.5mM的唾液酸到#1730培养物中。孵育持续72至96小时。然后,细胞被离心收集,并使用玻璃珠在确定的体积中机械地破坏。随后,将样品应用于薄层色谱(TLC),于硅胶60$F_{254}$上(Merck KGa A,Darmstadt,Germany)。使用丁醇:丙酮:乙酸:$H_2O$(35/35/7/23(v/v/v/v))的混合物为流动相。为检测分离的物质,用百里酚试剂(溶于95ml乙醇中的0.5g百里酚,加入5ml硫酸)浸泡TLC板并加热。

[0167] 结果总结在表5。在整个筛选过程中,鉴定出编码α-2,3-唾液酸转移酶的32种基因,从而生产3'-SL。19种酶合成了6'-SL并被描述为α-2,6-唾液酸转移酶。只有3种酶可以同时观察到α-2,3-以及α-2,6-唾液酸转移酶活性。因此,46种酶的表达不会导致唾液酸乳糖的形成。由于所描述的被广泛表征的唾液酸转移酶(例如SiaT1(Gilbert et al.,J Biol Che m.1996Nov 8;271(45):28271-6;Gilbert et al.,Eur J Biochem.1997 Oct 1;249(1):187-94)、SiaT6(Tsukamoto et al.,J Biochem.2008Feb;143(2):187-97)和SiaT11(Yamamoto et al.,J Biochem,1996Jul;120(1):104-10))的活性可被证实,因此所述筛选显得非常准确。关于产物的形成,实验设置允许对筛选的酶进行半定量比较。但为了更深入地了解它们的动力学性质,将3种可能具有最佳性能的α-2,3-和α-2,6-唾液酸转移酶应用于体外测定。

[0168]

| SiaT # | 关于唾液酸转移酶的体外生产唾液酸乳-*N*-四糖的相对效力 | | 唾液酸乳糖的体内生产 | SEQ ID NO: | |
|---|---|---|---|---|---|
| | LST-a | LST-b | | aa | nt |
| 1 | $2.98 \times 10^3$ | n.d. | 3'-SL | 38 | 5 |
| 2 | 749 | n.d. | 3'-SL | 43 | 10 |
| 3 | 605 | n.d. | | 45 | 12 |
| 4 | 654 | n.d. | | 44 | 11 |
| 5 | n.d. | 1 | | 66 | 33 |
| 6 | n.d. | 300 | 6'-SL | 62 | 29 |
| 7 | $2.25 \times 10^3$ | n.d. | | 40 | 7 |
| 8 | $4.00 \times 10^3$ | n.d. | | 36 | 3 |
| 9 | $8.39 \times 10^3$ | n.d. | 3'-SL | 35 | 2 |
| 10 | $3.18 \times 10^3$ | n.d. | 3'-SL, 6'-SL | 37 | 4 |
| 11 | n.d. | 13 | | 64 | 31 |
| 13 | 24 | n.d. | 3'-SL | 50 | 17 |
| 14 | 2 | n.d. | 3'-SL | 59 | 26 |
| 15 | 204 | n.d. | 3'-SL | 47 | 14 |
| 16 | 1 | n.d. | 3'-SL | 60 | 27 |
| 17 | $1.24 \times 10^3$ | n.d. | 3'-SL | 41 | 8 |
| 18 | n.d. | 303 | 6'-SL | 61 | 28 |
| 19 | n.d. | 157 | 6'-SL | 63 | 30 |
| 20 | $1.00 \times 10^4$ | n.d. | 3'-SL | 34 | 1 |
| 21 | 12 | n.d. | 3'-SL | 54 | 21 |
| 22 | 564 | n.d. | 3'-SL | 46 | 13 |
| 24 | 835 | n.d. | 3'-SL | 42 | 9 |
| 30 | 17 | n.d. | 3'-SL | 52 | 19 |
| 31 | 4 | n.d. | 3'-SL | 55 | 22 |
| 32 | 64 | n.d. | 3'-SL, 6'-SL | 48 | 15 |
| 34 | $2.34 \times 10^3$ | n.d. | 3'-SL | 39 | 6 |
| 37 | 40 | n.d. | 3'-SL | 49 | 16 |
| 39 | 4 | n.d. | 3'-SL | 56 | 23 |
| 41 | 3 | n.d. | 3'-SL | 57 | 24 |
| 42 | 3 | n.d. | 3'-SL | 58 | 25 |
| 43 | 21 | n.d. | 3'-SL | 51 | 18 |
| 51 | n.d. | 10 | 6'-SL | 65 | 32 |
| 73 | 15 | n.d. | 3'-SL | 53 | 20 |

[0169] 表5:唾液酸转移酶(SiaT)产物光谱的表征。分别用乳糖或LNT作为聚糖底物,通过体内或体外实验测定活性。通过薄层色谱定性鉴别唾液酸化乳糖。通过LC-MS/MS对LNT的唾液酸化产物进行定量。描述了LST-a或-b相对于最佳性能的酶的相对量(n.d.:未检出的)。

[0170] 实施例5:使用乳-N-四糖为受体底物鉴定和表征α-2,3-和α-2,6-唾液酸转移酶

[0171] 携带编码100种唾液酸转移酶的质粒的大肠杆菌BL21(DE3)在装有补充氨苄西林100μg $ml^{-1}$的20ml 2YT培养基的100ml的摇瓶中在30℃下生长。当培养物达到$OD_{600}$为0.1至0.3时,加入0.3mM IPTG诱导基因表达,孵育持续12至16小时。细胞被离心收集,并使用玻璃珠在确定体积的50mM Tris-HCl pH7.5中机械地破坏。蛋白质提取物被保存在冰上,直到测定开始。体外测定的总体积为25μl,包括50mM Tris-HCl pH7.5、5mM $MgCl_2$、10mM CMP-Neu5Ac和5mM LNT。该测定从加入3μl蛋白提取物开始,持续16小时。用质谱法测定产物的形

成。

[0172] 质谱分析法使用LC三重四极MS检测系统通过MRM(多反应监测)进行。在四极1中选择前体离子并进行分析,使用氩气为CID气体在碰撞池中发生碎片化,在四极3中选择碎片离子。用$H_2O$(LC/MS级)将培养上清液1:100稀释后,在携带有XBridge酰胺保护盒(XBridge Amide guard cartridge,3.5μm,2.1×10mm)(Waters,USA)的XBridge酰胺HPLC柱(3.5μm,2.1×50mm(Waters,USA))上进行乳糖、3'-唾液酸乳糖和6'-唾液酸乳糖的色谱分离。HPLC系统的柱箱温为50℃。流动相由乙腈:$H_2O$与10mM乙酸铵组成。在仪器中注入1μl样品;运行3.60min,流速为400μl/min。在ESI正电离模式下,用MRM分析3'-唾液酸乳糖和6'-唾液酸乳糖。在单位分辨率下操作质谱仪。唾液酸乳糖形成m/z 656.2[M+Na]的离子。在碰撞池中,唾液酸乳糖的前体离子进一步碎裂为碎片离子m/z 612.15、m/z 365.15和m/z 314.15。分别对每种分析物的碰撞能量、Q1和Q3预偏置(Pre Bias)进行优化。用$H_2O$(LC/MS级)分别将无颗粒生物催化反应或粗提取物1:50稀释后,在携带有XBridge酰胺保护盒(3.5μm,2.1×10mm)(Waters,USA)的XBridge酰胺HPLC柱(3.5μm,2.1×50mm)(Waters,USA)上进行乳糖、LNT-II、LNT和LST-a或-b的色谱分离。柱温箱在35℃下运行。流动相由乙腈:$H_2O$与0.1%氢氧化铵组成。向仪器中注入1μl样品;运行3.50min,流速为300μl/min。在ESI负电离模式下,用MRM分析乳糖、LNT-II、LNT以及LST-a和-b。在单位分辨率下操作质谱仪。乳糖形成m/z 341.00[M-H]的离子。乳糖的前体离子在碰撞池中进一步破碎成碎片离子m/z 179.15、m/z 161.15和m/z 101.05。LNT-II形成m/z 544.20[M-H]的离子。将LNT-II的前体离子进一步破碎成碎片离子m/z 382.10、m/z 161.00和m/z 112.90。LNT形成m/z 706.20[M-H]的离子。将LNT的前体离子进一步破碎成碎片离子m/z 382.10、m/z 202.10和m/z 142.00。LST-a和-b形成m/z 997.20[M-H]的离子。将LST-a和-b的前体离子进一步破碎成碎片离子m/z 290.15、m/z 202.15和m/z 142.15。分别对每种分析物的碰撞能量、Q1和Q3预偏置进行了优化。使用市售标准品(Carbosynth,Compton,UK)建立了定量方法。

[0173] 体外筛选结果总结在表5。有28种基因被鉴定为生产LST-a,而只有6种酶合成LST-b。因此,66种酶的表达不会导致LST-a或LST-b的形成。该测定被认为是准确的,因为SiaT1的活性(已被描述为唾液酸化LNT(Gilbert et al.,J Biol Chem.1996Nov 8;271(45):28271-6;Gilbert et al.,Eur J Biochem.19970ct 1;249(1):187-94))能够被证实。无论蛋白质过表达水平如何,选择生产最佳的唾液酸转移酶来测定$K_m$和$V_{max}$。

[0174] 实施例6:所选择的唾液酸转移酶的动力学性质的表征

[0175] 为了排列最佳性能的唾液酸转移酶,体外测定了供体和受体底物的$K_m$值。大肠杆菌BL21(DE3)菌株#287用于酶的过量生产。细胞在含有补充100μg $ml^{-1}$氨苄西林的100ml 2YT培养基的摇瓶中在30℃下孵育,直到达到$OD_{600}$为0.3。然后加入0.3mM IPTG,继续孵育12至16小时。细胞通过离心收集,并使用玻璃珠在确定体积的50mM Tris-HCl pH7.5中进行机械破坏。蛋白质提取物被保存在冰上,直到测定开始。进行体外测定的总体积为50μl,包括50mM Tris-HCl pH7.5、5mM $MgCl_2$和不同浓度的CMP-Neu5Ac(0.05-30mM)以及乳糖或LNT(0.1-50mM)。该测定从加入35至750μg蛋白质提取物开始。在30℃下孵育1至10分钟后,在95℃下灭活5分钟。采用质谱法测定产物形成。用SigmaPlot v12.5的酶动力学模块来评估数据,以计算$K_m$和$V_{max}$。

[0176] 在筛选过程中,对于LST-a生产表现最好的α-2,3-唾液酸转移酶似乎是SiaT8、

SiaT9和SiaT20。相反,在所测试的α-2,6-唾液酸转移酶中,SiaT6、SiaT18和SiaT19被观察到最有效地唾液酸化LNT。它们针对CMP-Neu5Ac和LNT以及乳糖的动力学参数如表6所示,只有SiaT20不遵循Michaelis-Menten动力学。

[0177]

| 活性 | SiaT# | CMP-Neu5Ac | | 乳糖 | | LNT | |
|---|---|---|---|---|---|---|---|
| | | $K_m$ [mM] | $V_{max}$ [μmol/min] | $K_m$ [mM] | $V_{max}$ [μmol/min] | $K_m$ [mM] | $V_{max}$ [μmol/min] |
| α2,3 | SiaT8 | 4.0 | 0.35 | 4.3 | 0.37 | 4.2 | 0.32 |
| | SiaT9 | 0.96 | 0.16 | 2.9 | 0.18 | 2.9 | 0.15 |
| α2,6 | SiaT6 | 2 | 0.16 | 5.6 | 0.20 | 5.5 | 0.13 |
| | SiaT18 | 2.8 | 0.21 | 7.6 | 0.21 | 5.2 | 0.14 |
| | SiaT19 | 1.1 | 0.09 | 3 | 0.09 | 2.9 | 0.04 |

[0178] 表6:以CMP-Neu5Ac为供体底物以及LNT和乳糖为受体底物,所选择的唾液酸转移酶的$K_m$和$V_{max}$。

[0179] 实施例7:用于筛选使用LNT作为受体底物的唾液酸转移酶的体内活性的乳-N-四糖生产菌株的产生

[0180] 使用大肠杆菌BL21(DE3)菌株#534构建了一种乳-N-四糖(LNT)生产菌株。将来自脑膜炎奈瑟氏菌(Neisseria meningitidis)MC58的β-1,3-N-乙酰葡糖胺基转移酶基因lgtA(登录号NP_274923)进行密码子优化以在大肠杆菌中表达并通过基因合成以合成方法制备。与编码来自大肠杆菌K-12次代菌株MG1655的半乳糖-1-磷酸尿苷基转移酶基因galT(登录号NP_415279)(类似地通过基因合成得到)一起,使用质粒pEcomar-lgtA-galT通过转座插入lgtA(SEQ ID NO:188)。为增强UDP-N-乙酰葡糖胺的从头合成,对编码L-谷氨酰胺:D-果糖-6-磷酸氨基转移酶的基因(glmS)、编码大肠杆菌K-12次代菌株MG1655的磷酸葡糖胺变位酶的基因(glmM)和编码大肠杆菌K-12次代菌株MG1655的N-乙酰葡糖胺-1-磷酸尿苷酰转移酶/葡糖胺-1-磷酸乙酰转移酶的基因(glmU)(登录号分别为NP_418185、NP_417643、NP_418186)进行密码子优化并通过基因合成获得。将操纵子glmUM克隆到组成型四环素启动子$P_{tet}$的控制下,同时将glmS克隆到组成型$P_{T5}$启动子的控制下。插入来自pEcomar-glmUM-glmS的转座子盒<$P_{tet}$-glmUM-$P_{T5}$-glmS-FRT-dhfr-FRT>,其侧翼为类水手元件Himar1转座酶特异性识别的反向末端重复,显示为乳-N-三糖II生产菌株。代谢工程化还包括转座子盒<$P_{tet}$-wbdO-$P_{T5}$-galE-FRT-cat-FRT>(SEQ ID NO:187)的基因组整合,所述转座子盒的侧翼为类水手元件Himar1转座酶特异性识别的反向末端重复,从pEcomar-wbdO-galE插入。为了防止N-乙酰神经氨酸的胞内降解,按照Datsenko and Wanner的方法(Proc.Natl.Acad.Sci.USA 97:6640-6645(2000)),从大肠杆菌BL21(DE3)菌株基因组中缺失nanKETA基因簇。为了向LNT的唾液酸化提供足够的供体底物(CMP-Neu5Ac),将唾液酸的摄取机制及其核苷酸活化能力是在大肠杆菌菌株中实现。如上所述,将nanT和neuA基因克隆作为操纵子克隆到组成型四环素启动子$P_{tet}$的控制下(使用引物neuA/nanT_1-6),并利用EZ-Tn5转座酶对得到的表达片段<$P_{tet}$-neuA-nanT-lox66-kan-lox72>进行整合,最终产生菌株#2130。

[0181] 实施例8:表达不同唾液酸转移酶的大肠杆菌BL21(DE3)#2130的分批发酵

[0182] 携带编码唾液酸转移酶SiaT9或SiaT19的表达质粒的大肠杆菌BL21(DE3)#2130细胞在装有补充2%(wt/vol)葡萄糖、5g/l $NH_4Cl$、氨苄西林100μg $ml^{-1}$、卡那霉素15μg $ml^{-1}$和

庆大霉素5μg $ml^{-1}$的25ml无机盐培养基(Samain et al.,J.Biotech.1999,72:33-47)的100ml摇瓶中在30℃下生长。当培养物达到$OD_{600}$为0.5至1时,添加3mM乳糖。孵育24小时后,通过加入0.3mM IPTG诱导唾液酸转移酶基因表达。同时,在培养物中加入3mM的唾液酸。孵育持续48小时。然后,细胞被离心收集,并使用玻璃珠在确定的体积中机械地破坏。随后,进行薄层色谱(TLC)以证实唾液酸乳-N-四糖-a和-b的细胞内形成。如图3所示,菌株#2130中siaT9或siaT19的表达分别导致LST-a和LST-b的形成。结果是经质谱法验证。

[0001] 序列表

[0002] <110> Jennewein Biotechnologie GmbH

[0003] <120> 唾液酸转移酶及其在生产唾液酸化低聚糖中的用途

[0004] <130> P 1703 WO

[0005] <160> 188

[0006] <170> PatentIn version 3.5

[0007] <210> 1

[0008] <211> 1410

[0009] <212> DNA

[0010] <213> 结肠弯曲杆菌(Campylobacter coli)

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[0014] ctgggcaaaa acatcaaagc ggccttttttc aatccgtatc cgttcctgca gcaataccat 180

[0015] accgcgaaac agctggtgtt caacaacgaa tacaaaatcg aaaacatctt ttgtagcacg 240

[0016] ttcaatctgc cgttcatcga aaaagataac ttcatcaaca aattttacga tttctttccg 300

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[0104] ctgaacctgc tgaatcattt tacccaggcg ggcggcgacc tgtttattgg tgaccattac 840

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[0106] gcaaaaaaca tcacgaatat cccggctaat atctctttcg aagtgctgat gatgaccggc 960

[0107] ctgctgccgg ataaagtcgg cggtgtggct agctctctgt acttcagtct gccgaaagaa 1020

[0108] aaaattagtc acatcatctt caccagcaac aaacaggtca aatcaaaaga agatgccctg 1080

[0109] aacaatccgt acgtgaaagt tatgcgtcgc ctgggtatta tcgatgaatc gcaagtgatc 1140

[0110] ttttgggaca gcctgaaaca gctgtaa 1167

[0111] <210> 5

[0112] <211> 1116

[0113] <212> DNA

[0114] <213> 脑膜炎奈瑟氏菌(Neisseria meningitidis)

[0115] <400> 5

[0116] atgggcctga aaaaagcctg cctgaccgtg ctgtgtctga tcgtgttttg cttcggcatc 60

[0117] ttttatacgt tcgatcgtgt gaaccagggt gaacgcaatg cagttagtct gctgaaagaa 120

[0118] aaactgttta acgaagaagg cgaaccggtg aatctgatct tctgttacac cattctgcaa 180

[0119] atgaaagttg ccgaacgtat tatggcacag catccgggtg aacgctttta tgtggttctg 240

[0120] atgagcgaaa accgtaacga aaaatacgat tactacttca accagatcaa agataaagcg 300

[0121] gaacgcgcct atttctttca cctgccgtac ggcctgaaca aaagttttaa tttcattccg 360

[0122] acgatggcgg aactgaaagt gaaaagcatg ctgctgccga aagttaaacg tatctatctg 420

[0123] gcaagcctgg aaaaagtgtc tattgcggcc tttctgagca cctacccgga tgcggaaatc 480

[0124] aaaaccttcg atgatggcac gggtaatctg attcagagct ctagttatct gggcgatgaa 540

[0125] ttttctgtta acggtacgat caaacgtaat ttcgcccgca tgatgatcgg tgattggtct 600

[0126] attgcgaaaa cccgcaacgc cagtgatgaa cattacacga tcttcaaagg cctgaaaaac 660

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[0132] ttcagcggcg ccgcactgac catgaaagat tttccgaacg tgcacgttta tgcactgaaa 1020

[0133] ccggcgtctc tgccggaaga ttattggctg aaaccggtgt acgcgctgtt tacccagagt 1080

[0134] ggtattccga tcctgacgtt cgatgataaa aattaa 1116

[0135] <210> 6

[0136] <211> 852

[0137] <212> DNA

[0138] <213> 多杀性巴氏杆菌(Pasteurella multocida)

[0139] <400> 6

[0140] atggataaat ttgcagaaca tgaaattccg aaagcagtga tcgttgctgg caacggtgaa 60

[0141] agtctgtccc agattgatta tcgtctgctg ccgaaaaact acgacgtctt ccgttgcaac 120

[0142] caattctact tcgaagaacg ctacttcctg ggcaataaaa tcaaagccgt gtttttcacc 180

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[0146] ctgaccgctt tcgatgttta tctgcgttac aaagaactgt atgaaaatca gcgcattacg 420

[0147] agcggtgttt acatgtgcgc tgtcgcgatc gccatgggct ataccgatat ttacctgacg 480

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[0152] gaaaccacgt tcgtcgcccc gctgaaagaa aactatatta atgacatcct gctgccgccg 780

[0153] cactttgtct atgaaaaact gggcgtggat aaactggcgg ccgcactgga acatcaccat 840

[0154] caccatcact aa 852

[0155] <210> 7

[0156] <211> 1158

[0157] <212> DNA

[0158] <213> 达可马巴斯德氏菌(Pasteurella dagmatis)

[0159] <400> 7

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[0161] aaagaaagcg aagacaaaga aaccgcacgt atttttggct tctctcgctt taaactgccg 120

[0162] gaaaaaatca cggaacagta caacaacatc catttcgtgg aaatcaaaaa caatcgtccg 180

[0163] acggaagata ttttcaccat cctggaccag tacccggaaa aactggaact ggatctgcat 240

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[0166] gatctggaaa aagaagaaaa caaagacatc aaaagtgcga tcaaaaaagc cgaaaaacag 420

[0167] ctgtccgatt atctgctgac gggtaaaatt aactttgaca atccgaccct ggcacgctac 480

[0168] gtttggcagt cacaatatcc ggtcaaatac catttcctgt cgacggaata ttttgaaaaa 540

[0169] gctgaattcc tgcagccgct gaaaacctat ctggcgggca aataccaaaa aatggattgg 600

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[0173] ctgaaacatt ttacccacag cgaaggcgac ctgtttatcg gtgaccagta caaaatctac 840

[0174] ttcaaaggcc atccgcgcgg cggtgatatt aacgactata tcctgaaaca cgcaaaagat 900

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[0179] tctctgaaac aactgtaa 1158

[0180] <210> 8

[0181] <211> 1173

[0182] <212> DNA

[0183] <213> 明亮发光杆菌(Photobacterium phosphoreum)

[0184] <400> 8

[0185] atgggctgta actccgatag caaacacaat aacagtgatg gcaatattac caaaaacaaa 60

[0186] acgatcgaag tctatgtgga ccgtgcgacc ctgccgacga ttcagcaaat gacccagatc 120

[0187] atcaacgaaa atagcaacaa caaaaaactg atttcatggt cgcgttaccc gatcaatgat 180

[0188] gaaacgctgc tggaatcaat taatggctcg tttttcaaaa accgcccgga actgatcaaa 240

[0189] agtctggatt ccatgattct gaccaacgaa attaagaaag tgatcatcaa cggtaacacg 300

[0190] ctgtgggcag ttgacgtggt taatattatc aaaagcattg aagctctggg caagaaaacc 360

[0191] gaaatcgaac tgaacttcta tgatgacggt tctgcggaat atgtgcgtct gtacgatttt 420

[0192] agccgcctgc cggaatctga acaggaatac aaaattagcc tgtctaaaga taacattcag 480

[0193] agcagcatca acggcaccca accgttcgac aacagcatcg aaaacatcta cggtttctct 540

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[0196] accacgttca attcccagca gaaaaacaaa ttttacaact tcaccggctt caacccggaa 720

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[0198] tccggcaccg ccaccgcaga acagcaaatt gatatcctga ccgaagccaa aaaaccggac 840

[0199] tcaccgatta tcaccaacag cattcagggc ctggacctgt ttttcaaagg tcatccgtct 900

[0200] gcgacctata accagcaaat tatcgacgcc cacaacatga tcgaaatcta caacaaaatc 960

[0201] ccgttcgaag cactgatcat gaccgatgca ctgccggacg ctgttggcgg tatgggtagt 1020

[0202] tccgtctttt tctcactgcc gaataccgtc gaaaacaaat tcattttcta taaatcggat 1080

[0203] acggacattg aaaacaatgc tctgatccag gttatgatcg aactgaatat cgtgaaccgc 1140

[0204] aatgatgtga aactgattag tgacctgcaa taa 1173

[0205] <210> 9

[0206] <211> 1254

[0207] <212> DNA

[0208] <213> 副鸡禽杆菌(Avibacterium paragallinarum)

[0209] <400> 9

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[0211] atggaaatct acctggacta tgcgtcgctg ccgagcctga acatgatcct gaacctggtt 120

[0212] gaaaacaaaa acaacgaaaa agtcgaacgt attatcggct tcgaacgctt tgatttcaac 180

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[0215] gacctgatta tccataccaa tctggatcac tcagttcgtt cgctgctgag catctttaaa 360

[0216] accctgagtc cgctgttcca taaaatcaac atcgaaaaac tgtacctgta cgatgacggc 420

[0217] agcggtaact atgttgatct gtaccagcac cgccaagaaa atatttctgc gattctgatc 480

[0218] gaagcccaga aaaaactgaa agacgcgctg gaaaatcgtg aaacggatac cgacaaactg 540

[0219] catagcctga cgcgctatac ctggcacaaa atctttccga cggaatatat cctgctgcgt 600

[0220] ccggattacc tggatattga cgaaaaaatg caaccgctga aacatttcct gagcgatacc 660

[0221] atcgtgtcta tggacctgtc tcgctttagt catttctcca aaaaccagaa agaactgttt 720

[0222] ctgaaaatca cgcacttcga tcaaaacatc ttcaacgaac tgaacatcgg caccaaaaac 780

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[0225] aaattctacc tgggtaacga tatcaaaatc tttttcaaag gccacccgaa aggtgatgac 960

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[0227] gaagttctga tgatgacgaa tagtctgccg gattatgtcg gcggtattat gagtaccgtg 1080

[0228] tacttttccc tgccgccgaa aaatattgat aaagtggttt tcctgggttc cgaaaaaatc 1140

[0229] aaaaacgaaa acgacgccaa atcacagacc ctgtcgaaac tgatgctgat gctgaacgtc 1200

[0230] atcacgccgg aacagatttt ctttgaagaa atgccgaacc cgattaactt ttaa 1254

[0231] <210> 10

[0232] <211> 1293

[0233] <212> DNA

[0234] <213> 空肠弯曲杆菌(Campylobacter jejuni)

[0235] <400> 10

[0236] atgacccgca cccgtatgga aaacgaactg attgtgagca aaaacatgca gaacattatt 60

[0237] atcgccggta acggtccgag cctgaaaaat attaactata aacgtctgcc gcgcgaatac 120

[0238] gatgtgttcc gttgcaacca gttctacttc gaagacaaat actacctggg caagaaaatt 180

[0239] aaagccgtgt ttttcaatcc gggcgtgttt ctgcaacaat atcataccgc aaaacagctg 240

[0240] attctgaaaa acgaatacga aatcaaaaac atcttttgta gcaccttcaa tctgccgttt 300

[0241] atcgaatcta acgatttcct gcaccaattt tataactttt tcccggacgc taaactgggc 360

[0242] tacgaagtca tcgaaaacct gaaagaattt tacgcgtaca tcaaatacaa cgaaatctac 420

[0243] ttcaacaaac gcatcacctc tggcgtgtat atgtgcgcga ttgccatcgc actgggttat 480

[0244] aaaacgattt acctgtgtgg catcgatttc tatgaaggtg acgttattta cccgtttgaa 540

[0245] gcaatgagta ccaacattaa aacgatcttc ccgggtatca aagatttcaa accgagtaac 600

[0246] tgccattcca aagaatatga catcgaagcg ctgaaactgc tgaaaagcat ctacaaagtt 660

[0247] aacatctacg ccctgtgtga tgacagtatt ctggcaaatc atttcccgct gtccattaac 720

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[0252] ctgagctata aactgggcca agccctgatt atcaatagca aatctgtcct gggtttcctg 1020

[0253] tctctgccgt ttattatcct gtcaattgtg atctcgcaca aacaggaaca aaaagcgtat 1080

[0254] aaattcaaag tgaagaaaaa cccgaacctg gcactgccgc cgctggaaac ctatccggat 1140

[0255] tacaacgaag ccctgaaaga aaaagaatgc ttcacgtaca aactgggcga agaatttatc 1200

[0256] aaagcaggta aaaactggta tggcgaaggt tacatcaaat ttatcttcaa agatgttccg 1260

[0257] cgtctgaaac gtgaatttga aaaaggcgaa taa 1293

[0258] <210> 11

[0259] <211> 1188

[0260] <212> DNA

[0261] <213> 蓖麻毒素螺杆菌(Heliobacter acinonychis)

[0262] <400> 11

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[0264] gcgttgttcc cgaaagactt tgatgtattc cgatgtaatc aattctactt cgaggacaaa 120

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[0266] atgaagatca ctaaagccat agtcaaaaac ggggaatatc acccggacca catatattgc 240

[0267] acacatgtcg aaccgtacgg ttacgttaac ggaaaccagc aactcatgca agagtacctg 300

[0268] gaaaaacatt ttgtgggagt ccgaagcacg tacgcatacc tgaaagatct agagccattc 360

[0269] tttattctgc acagtaagta tcgcaacttc tacgaccagc acttcacaac gggcatcatg 420

[0270] atgctactgg tggccatcca attgggatac aaagaaatat acctgtgcgg aatagacttc 480

[0271] tacgaaaacg gattcggaca tttctacgag aaccaagggg gattctttga agaggatagc 540

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[0278] gagcgtcaaa aagaattaga gaggcagaag gagttagaaa ggcagaaaga actggagaga 960

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[0283] <210> 12

[0284] <211> 783

[0285] <212> DNA

[0286] <213> 空肠弯曲杆菌(Campylobacter jejuni)

[0287] <400> 12

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[0289] cgtctgccga acgattttga tgtgtttcgc tgcaaccagt tttatttcga agataaatat 120

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[0292] aactataacc aggcgcatct ggaaaacgaa aactttgtga aaaccttcta cgattatttt 300

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[0301] taa 783
[0302] <210> 13
[0303] <211> 897
[0304] <212> DNA
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[0322] <210> 14
[0323] <211> 888
[0324] <212> DNA
[0325] <213> 杜克雷嗜血杆菌(Haemophilus ducreyi)
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[0328] aataaaaatg tcgaagacgt gttcttcatt tttgaaaaag gcgcgatgcc gaacaatctg 120
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[0335] aaaaccgcca cggaaaaaac gaaaattctg agctttttcg aaatcgatat gcaggaaatt 540

[0336] tcagttatca aaaaccgctc gtttgtcctg tatacccaac cgctgtcaga agataaactg 600

[0337] ctgacggaag cggaaaaaat tgacatctat cgtaccattc tgacgaaata caaccattcg 660

[0338] cagaccgtta tcaaaccgca cccgcgcgat aaaacggact ataaacaact gtttccggat 720

[0339] gcctatgtca tgaaaggcac ctacccgagt gaactgctga cgctgctggg tgtcaacttc 780

[0340] aacaaagtga tcaccctgtt ttccacggcg gtcttcgatt atccgaaaga aaaaatcgac 840

[0341] ttctacggca ccgcggtgca tccgaaactg ctggatttct ttgactaa 888

[0342] <210> 15

[0343] <211> 1467

[0344] <212> DNA

[0345] <213> 别样杆菌属种(Alistipes sp.)

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[0355] gaagttacca ccgcagctga tcgtaccgcc gatgcaaccc aggccgaaat ggaagcaatg 540

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[0372] <210> 16

[0373] <211> 876

[0374] <212> DNA

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[0414] <211> 1233
[0415] <212> DNA
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[0439] <210> 19

[0440] <211> 1221

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[0442] <213> 副溶血嗜血杆菌(Haemophilus parahaemolyticus)

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[0465] <210> 20

[0466] <211> 903

[0467] <212> DNA

[0468] <213> 睡眠嗜血杆菌(Haemophilus somnus)

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[0473] aaattctggc gtctgtacta caaactgtac ttctaccgtt tcaaaattga ccgcatcccg 240

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[0485] taa 903

[0486] <210> 21

[0487] <211> 1146

[0488] <212> DNA

[0489] <213> 哈氏弧菌(Vibrio harveyi)

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[0542] <211> 1452

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[0571] <210> 24

[0572] <211> 1206

[0573] <212> DNA

[0574] <213> 猪放线杆菌(Actinobacillus suis)

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[0596] tcgtaa 1206

[0597] <210> 25

[0598] <211> 1206

[0599] <212> DNA

[0600] <213> 荚膜放线杆菌(Actinobacillus capsulatus)

[0601] <400> 25

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[0621] caagcaatgc tggaaatgca actggtctcg caagaacaag tctactactg ctcgctgtcg 1200

[0622] tcgtaa 1206

[0623] <210> 26

[0624] <211> 936

[0625] <212> DNA

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[0627] <400> 26

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[0644] <210> 27
[0645] <211> 1200
[0646] <212> DNA
[0647] <213> 杜克雷嗜血杆菌(Haemophilus ducreyi)
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[0669] <210> 28
[0670] <211> 1494
[0671] <212> DNA

[0672] <213> 鳀鱼发光杆菌(Photobacterium leiognathi)

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[0676] tcctggattc agacgtgcgg caccccgatc ctgaaagatg acgaaaaata ttcactgtcg 180

[0677] tttgatttcg tcgccccgga actggatcag gacgaaaaat tctgtttcga atttaccggc 240

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[0699] <210> 29

[0700] <211> 1497

[0701] <212> DNA

[0702] <213> 发光杆菌属种(Photobacterium sp.)

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[0729] <210> 30

[0730] <211> 1449

[0731] <212> DNA

[0732] <213> 鳐鱼发光杆菌(Photobacterium leiognathi)

[0733] <400> 30

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[0759] <210> 31

[0760] <211> 2028

[0761] <212> DNA

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[0819] aaactgagtg actcccagca aaccctgttt ctggatatcg ttggcttcga caaagaacag 1020

[0820] ctgcaacaac agtattcaca atcgccgctg ccgaatttta tttttaccgg caccaccacc 1080

[0821] tgggcgggcg gtgaaacgaa agaatattac gcccaacagc aagtgaacgt tattaacaat 1140

[0822] gccatcaatg aaaccagccc gtattacctg ggcaaagatt acgacctgtt tttcaaaggt 1200

[0823] catccggcag gcggtgtgat caacgatatt atcctgggca gttttccgga catgattaat 1260

[0824] atcccggcta aaatttcctt cgaagtgctg atgatgaccg atatgctgcc ggacacggtt 1320

[0825] gcaggtatcg ctagctctct gtattttacc attccggcgg ataaagtgaa ctttatcgtt 1380

[0826] ttcacgagtt ccgatacgat taccgaccgt gaagaagccc tgaaaagccc gctggtccag 1440

[0827] gtgatgctga ccctgggcat cgtcaaagaa aaagatgtgc tgttctgggc agacctgccg 1500

[0828] gactgctcgt ctggtgtgtg tatcgacaaa taa 1533

[0829] <210> 33

[0830] <211> 1269

[0831] <212> DNA

[0832] <213> 蓖麻毒素螺杆菌(Heliobacter acinonychis)

[0833] <400> 33

[0834] atggggacca ttaaaaagcc cttaatcata gcaggaaatg gtccatcaat taaggaccta 60

[0835] gactatgctt tatttccaaa agacttcgat gtctttcgct gcaaccagtt ttacttcgag 120

[0836] gataaatatt acctaggacg cgaaataaaa ggagtgttct ttaacccttg tgtattaagc 180

[0837] agtcaaatgc aaacagtgca ataccttatg gacaatggcg aatatagcat agaacgcttc 240

[0838] ttttgcagtg tttcaacaga tcgccacgat tttgatgggg attaccaaac gattttaccg 300

[0839] gtagacggtt atttaaaagc acactatccg ttcgtctgcg atacattcag cttattcaaa 360

[0840] ggtcacgaag aaatcttaaa acacgtgaaa taccacctga aaacgtacag caaagaactt 420

[0841] agtgcgggtg tcttaatgtt attgagtgca gtggtattag gatacaaaga aatataccta 480

[0842] gtaggaatcg acttcggcgc ctcatcttgg gggcacttct atgacgaaag ccaatcccaa 540

[0843] cactttagca atcacatggc agattgtcac aatatctatt acgacatgct gactatttgt 600

[0844] ctctgtcaaa agtatgcaaa attgtacgca ttagcaccca attcaccatt atcacatttg 660

[0845] cttacactaa atccacaggc caaataccca tttgaactat tagataaacc tatcgggtat 720

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[0847] gaagagaagt tgcttgagtt caaaaacata gaagagaaac tcttagagtt caagaatatt 840

[0848] gaagagaaac tattagaatt taaaaacatc gaggaaaaac ttttggagtt caaaaatata 900

[0849] gaagagaaac tcctagagtt caagaacatt gaggaaaagt tgcttgagtt caaaaatatt 960

[0850] gaggaaaagt tgctcgaatt taagaatatc gaggaaaaac ttttggaatt taagaacata 1020

[0851] gaagaaaagt tactcgaatt taaaaacatt gaagagaaac tattggaatt taaaaatata 1080

[0852] gaggaaaagt tacttgagtt caaaaacata gaggaaaagt tacttgaatt taagaacata 1140

[0853] gaagagaaac ttctcgcaag ccgactgaac aacattctac gtaaaatcaa gcggaaaata 1200

[0854] cttccattct tttggggcgg aggtgtaacc ccaacattaa aagttagttt ccgttgggga 1260

[0855] gctgcataa 1269

[0856] <210> 34

[0857] <211> 469

[0858] <212> PRT

[0859] <213> 结肠弯曲杆菌(Campylobacter coli)

[0860] <400> 34

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[0863] Asn Tyr Gln Arg Leu Pro Lys Glu Tyr Asp Ile Phe Arg Cys Asn Gln

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[0865] Phe Tyr Phe Glu Asp Lys Tyr Tyr Leu Gly Lys Asn Ile Lys Ala Ala

[0866] 35 40 45

[0867] Phe Phe Asn Pro Tyr Pro Phe Leu Gln Gln Tyr His Thr Ala Lys Gln

[0868] 50 55 60

[0869] Leu Val Phe Asn Asn Glu Tyr Lys Ile Glu Asn Ile Phe Cys Ser Thr

[0870] 65 70 75 80

[0871] Phe Asn Leu Pro Phe Ile Glu Lys Asp Asn Phe Ile Asn Lys Phe Tyr

[0872] 85 90 95

[0873] Asp Phe Phe Pro Asp Ala Lys Leu Gly His Lys Ile Ile Glu Asn Leu

[0874] 100 105 110

[0875] Lys Glu Phe Tyr Ala Tyr Ile Lys Tyr Asn Glu Ile Tyr Leu Asn Lys

[0876] 115 120 125

[0877] Arg Ile Thr Ser Gly Ile Tyr Met Cys Ala Ile Ala Ile Ala Leu Gly

[0878] 130 135 140

[0879] Tyr Lys Asn Ile Tyr Leu Cys Gly Ile Asp Phe Tyr Glu Gly Glu Thr

[0880] 145 150 155 160

[0881] Ile Tyr Pro Phe Lys Ala Met Ser Lys Asn Ile Lys Lys Ile Phe Pro

[0882] 165 170 175

[0883] Trp Ile Lys Asp Phe Asn Pro Ser Asn Phe His Ser Lys Glu Tyr Asp

[0884] 180 185 190

[0885] Ile Glu Ile Leu Lys Leu Leu Glu Ser Ile Tyr Lys Val Asn Ile Tyr

[0886] 195 200 205

[0887] Ala Leu Cys Asp Asn Ser Ala Leu Ala Asn Tyr Phe Pro Leu Leu Val

[0888] 210 215 220

[0889] Asn Thr Asp Asn Ser Phe Val Leu Glu Asn Lys Ser Asp Asp Cys Ile

[0890] 225 230 235 240

[0891] Asn Asp Ile Leu Leu Thr Asn Asn Thr Pro Gly Ile Asn Phe Tyr Lys

[0892] 245 250 255

[0893] Ser Gln Ile Gln Val Asn Asn Thr Glu Ile Leu Leu Leu Asn Phe Gln

[0894] 260 265 270

[0895] Asn Met Ile Ser Ala Lys Glu Asn Glu Ile Ser Asn Leu Asn Lys Ile

[0896] 275 280 285

[0897] Leu Gln Asp Ser Tyr Lys Thr Ile Asn Thr Lys Glu Asn Glu Ile Ser

[0898] 290 295 300

[0899] Asn Leu Asn Lys Ile Leu Gln Asp Ser Tyr Lys Thr Ile Asn Thr Lys

[0900] 305 310 315 320

[0901] Glu Asn Glu Ile Ser Asn Leu Asn Lys Ile Leu Gln Asp Lys Asp Lys

[0902] 325 330 335

[0903] Leu Leu Ile Val Lys Glu Asn Leu Leu Asn Phe Lys Ser Arg His Gly

[0904] 340 345 350

[0905] Lys Ala Lys Phe Arg Ile Gln Asn Gln Leu Ser Tyr Lys Leu Gly Gln

[0906] 355 360 365

[0907] Ala Met Met Val Asn Ser Lys Ser Leu Leu Gly Tyr Ile Arg Met Pro

[0908] 370 375 380

[0909] Phe Val Leu Ser Tyr Ile Lys Asp Lys His Lys Gln Glu Gln Lys Ile

[0910] 385 390 395 400

[0911] Tyr Gln Glu Lys Ile Lys Lys Asp Pro Ser Leu Thr Leu Pro Pro Leu

[0912] 405 410 415

[0913] Glu Asp Tyr Pro Asp Tyr Lys Glu Ala Leu Lys Glu Lys Glu Cys Leu

[0914] 420 425 430

[0915] Thr Tyr Arg Leu Gly Gln Thr Leu Ile Lys Ala Asp Gln Glu Trp Tyr

[0916] 435 440 445

[0917] Lys Gly Gly Tyr Val Lys Met Trp Phe Glu Ile Lys Lys Leu Lys Lys

[0918] 450 455 460

[0919] Glu Tyr Lys Lys Lys

[0920] 465

[0921] <210> 35

[0922] <211> 381

[0923] <212> PRT

[0924] <213> 弧菌属种(Vibrio sp.)

[0925] <400> 35

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[0928] Tyr Val Asp Arg Ala Thr Leu Pro Thr Ile Gln Gln Met Thr Lys Ile

[0929] 20 25 30

[0930] Val Ser Gln Lys Thr Ser Asn Lys Lys Leu Ile Ser Trp Ser Arg Tyr

[0931] 35 40 45

[0932] Pro Ile Thr Asp Lys Ser Leu Leu Lys Lys Ile Asn Ala Glu Phe Phe

[0933] 50 55 60

[0934] Lys Glu Gln Phe Glu Leu Thr Glu Ser Leu Lys Asn Ile Ile Leu Ser

[0935] 65 70 75 80

[0936] Glu Asn Ile Asp Asn Leu Ile Ile His Gly Asn Thr Leu Trp Ser Ile

[0937] 85 90 95

[0938] Asp Val Val Asp Ile Ile Lys Glu Val Asn Leu Leu Gly Lys Asn Ile

[0939] 100 105 110

[0940] Pro Ile Glu Leu His Phe Tyr Asp Asp Gly Ser Ala Glu Tyr Val Arg

[0941] 115 120 125

[0942] Ile Tyr Glu Phe Ser Lys Leu Pro Glu Ser Glu Gln Lys Tyr Lys Thr

[0943] 130 135 140

[0944] Ser Leu Ser Lys Asn Asn Ile Lys Phe Ser Ile Asp Gly Thr Asp Ser

[0945] 145 150 155 160

[0946] Phe Lys Asn Thr Ile Glu Asn Ile Tyr Gly Phe Ser Gln Leu Tyr Pro

[0947] 165 170 175

[0948] Thr Thr Tyr His Met Leu Arg Ala Asp Ile Phe Asp Thr Thr Leu Lys

[0949] 180 185 190

[0950] Ile Asn Pro Leu Arg Glu Leu Leu Ser Asn Asn Ile Lys Gln Met Lys

[0951] 195 200 205

[0952] Trp Asp Tyr Phe Lys Asp Phe Asn Tyr Lys Gln Lys Asp Ile Phe Tyr

[0953] 210 215 220

[0954] Ser Leu Thr Asn Phe Asn Pro Lys Glu Ile Gln Glu Asp Phe Asn Lys

[0955] 225 230 235 240

[0956] Asn Ser Asn Lys Asn Phe Ile Phe Ile Gly Ser Asn Ser Ala Thr Ala

[0957] 245 250 255

[0958] Thr Ala Glu Glu Gln Ile Asn Ile Ile Ser Glu Ala Lys Lys Glu Asn

[0959] 260 265 270

[0960] Ser Ser Ile Ile Thr Asn Ser Ile Ser Asp Tyr Asp Leu Phe Phe Lys

[0961] 275 280 285

[0962] Gly His Pro Ser Ala Thr Phe Asn Glu Gln Ile Ile Asn Ala His Asp

[0963] 290 295 300

[0964] Met Ile Glu Ile Asn Asn Lys Ile Pro Phe Glu Ala Leu Ile Met Thr

[0965] 305 310 315 320

[0966] Gly Ile Leu Pro Asp Ala Val Gly Gly Met Gly Ser Ser Val Phe Phe
[0967] 325 330 335
[0968] Ser Ile Pro Lys Glu Val Lys Asn Lys Phe Val Phe Tyr Lys Ser Gly
[0969] 340 345 350
[0970] Thr Asp Ile Glu Asn Asn Ser Leu Ile Gln Val Met Leu Lys Leu Asn
[0971] 355 360 365
[0972] Leu Ile Asn Arg Asp Asn Ile Lys Leu Ile Ser Asp Ile
[0973] 370 375 380
[0974] <210> 36
[0975] <211> 390
[0976] <212> PRT
[0977] <213> 发光杆菌属种(Photobacterium sp.)
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[0981] Thr Lys Asn Lys Thr Ile Glu Val Tyr Val Asp Arg Ala Thr Leu Pro
[0982] 20 25 30
[0983] Thr Ile Gln Gln Met Thr Gln Ile Ile Asn Glu Asn Ser Asn Asn Lys
[0984] 35 40 45
[0985] Lys Leu Ile Ser Trp Ser Arg Tyr Pro Ile Asn Asp Glu Glu Leu Leu
[0986] 50 55 60
[0987] Glu Ser Ile Asn Gly Ser Phe Phe Lys Asn Asn Ser Glu Leu Ile Lys
[0988] 65 70 75 80
[0989] Ser Leu Asp Ser Met Ile Leu Thr Asn Asp Ile Lys Lys Val Ile Ile
[0990] 85 90 95
[0991] Asn Gly Asn Thr Leu Trp Ala Ala Asp Val Val Asn Ile Ile Lys Ser
[0992] 100 105 110
[0993] Ile Glu Ala Phe Gly Lys Lys Thr Glu Ile Glu Leu Asn Phe Tyr Asp
[0994] 115 120 125
[0995] Asp Gly Ser Ala Glu Tyr Val Arg Leu Tyr Asp Phe Ser Lys Leu Pro
[0996] 130 135 140
[0997] Glu Ser Glu Gln Glu Tyr Lys Ile Ser Leu Ser Lys Asp Asn Ile Leu
[0998] 145 150 155 160
[0999] Ser Ser Ile Asn Gly Thr Gln Pro Phe Glu Asn Val Val Glu Asn Ile
[1000] 165 170 175
[1001] Tyr Gly Phe Ser Gln Leu Tyr Pro Thr Thr Tyr His Met Leu Arg Ala
[1002] 180 185 190
[1003] Asp Ile Phe Glu Thr Asn Leu Pro Leu Arg Ser Leu Lys Gly Val Leu
[1004] 195 200 205
[1005] Ser Asn Asn Ile Lys Gln Met Lys Trp Asp Tyr Phe Lys Thr Phe Asn
[1006] 210 215 220
[1007] Ser Gln Gln Lys Asp Lys Phe Tyr Asn Phe Thr Gly Phe Asn Pro Asp

[1008] 225 230 235 240
[1009] Glu Ile Met Glu Gln Tyr Lys Ala Ser Pro Asn Lys Asn Phe Ile Phe
[1010] 245 250 255
[1011] Val Gly Thr Asn Ser Gly Thr Ala Thr Ala Glu Gln Gln Ile Asp Ile
[1012] 260 265 270
[1013] Leu Thr Glu Ala Lys Asn Pro Asn Ser Pro Ile Ile Thr Lys Ser Ile
[1014] 275 280 285
[1015] Gln Gly Phe Asp Leu Phe Phe Lys Gly His Pro Ser Ala Thr Tyr Asn
[1016] 290 295 300
[1017] Lys Gln Ile Ile Asp Ala His Asn Met Ile Glu Ile Tyr Asn Lys Ile
[1018] 305 310 315 320
[1019] Pro Phe Glu Ala Leu Ile Met Thr Asp Ala Leu Pro Asp Ala Val Gly
[1020] 325 330 335
[1021] Gly Met Gly Ser Ser Val Phe Phe Ser Leu Pro Asn Thr Val Glu Asn
[1022] 340 345 350
[1023] Lys Phe Ile Phe Tyr Lys Ser Asp Thr Asp Ile Glu Asn Asn Ala Leu
[1024] 355 360 365
[1025] Ile Gln Val Met Ile Glu Leu Asn Ile Val Asn Arg Asn Asp Val Lys
[1026] 370 375 380
[1027] Leu Ile Ser Asp Leu Gln
[1028] 385 390
[1029] <210> 37
[1030] <211> 388
[1031] <212> PRT
[1032] <213> 多杀性巴氏杆菌(Pasteurella multocida)
[1033] <400> 37
[1034] Met Lys Thr Ile Thr Leu Tyr Leu Asp Pro Ala Ser Leu Pro Ala Leu
[1035] 1 5 10 15
[1036] Asn Gln Leu Met Asp Phe Thr Gln Asn Asn Glu Asp Lys Thr His Pro
[1037] 20 25 30
[1038] Arg Ile Phe Gly Leu Ser Arg Phe Lys Ile Pro Asp Asn Ile Ile Thr
[1039] 35 40 45
[1040] Gln Tyr Gln Asn Ile His Phe Val Glu Leu Lys Asp Asn Arg Pro Thr
[1041] 50 55 60
[1042] Glu Ala Leu Phe Thr Ile Leu Asp Gln Tyr Pro Gly Asn Ile Glu Leu
[1043] 65 70 75 80
[1044] Asp Ile His Leu Asn Ile Ala His Ser Val Gln Leu Ile Arg Pro Ile
[1045] 85 90 95
[1046] Leu Ala Tyr Arg Phe Lys His Leu Asp Arg Val Ser Ile Gln Arg Leu
[1047] 100 105 110
[1048] Asn Leu Tyr Asp Asp Gly Ser Met Glu Tyr Val Asp Leu Glu Lys Glu
[1049] 115 120 125

[1050] Glu Asn Lys Asp Ile Ser Ala Glu Ile Lys Gln Ala Glu Lys Gln Leu
[1051] 130 135 140
[1052] Ser His Tyr Leu Leu Thr Gly Lys Ile Lys Phe Asp Asn Pro Thr Ile
[1053] 145 150 155 160
[1054] Ala Arg Tyr Val Trp Gln Ser Ala Phe Pro Val Lys Tyr His Phe Leu
[1055] 165 170 175
[1056] Ser Thr Asp Tyr Phe Glu Lys Ala Glu Phe Leu Gln Pro Leu Lys Glu
[1057] 180 185 190
[1058] Tyr Leu Ala Glu Asn Tyr Gln Lys Met Asp Trp Thr Ala Tyr Gln Gln
[1059] 195 200 205
[1060] Leu Thr Pro Glu Gln Gln Ala Phe Tyr Leu Thr Leu Val Gly Phe Asn
[1061] 210 215 220
[1062] Asp Glu Val Lys Gln Ser Leu Glu Val Gln Gln Ala Lys Phe Ile Phe
[1063] 225 230 235 240
[1064] Thr Gly Thr Thr Thr Trp Glu Gly Asn Thr Asp Val Arg Glu Tyr Tyr
[1065] 245 250 255
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[1067] 260 265 270
[1068] Asp Leu Phe Ile Gly Asp His Tyr Lys Ile Tyr Phe Lys Gly His Pro
[1069] 275 280 285
[1070] Arg Gly Gly Glu Ile Asn Asp Tyr Ile Leu Asn Asn Ala Lys Asn Ile
[1071] 290 295 300
[1072] Thr Asn Ile Pro Ala Asn Ile Ser Phe Glu Val Leu Met Met Thr Gly
[1073] 305 310 315 320
[1074] Leu Leu Pro Asp Lys Val Gly Gly Val Ala Ser Ser Leu Tyr Phe Ser
[1075] 325 330 335
[1076] Leu Pro Lys Glu Lys Ile Ser His Ile Ile Phe Thr Ser Asn Lys Gln
[1077] 340 345 350
[1078] Val Lys Ser Lys Glu Asp Ala Leu Asn Asn Pro Tyr Val Lys Val Met
[1079] 355 360 365
[1080] Arg Arg Leu Gly Ile Ile Asp Glu Ser Gln Val Ile Phe Trp Asp Ser
[1081] 370 375 380
[1082] Leu Lys Gln Leu
[1083] 385
[1084] <210> 38
[1085] <211> 371
[1086] <212> PRT
[1087] <213> 脑膜炎奈瑟氏菌(Neisseria meningitidis)
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[1090] 1 5 10 15
[1091] Cys Phe Gly Ile Phe Tyr Thr Phe Asp Arg Val Asn Gln Gly Glu Arg

[1092] 20 25 30
[1093] Asn Ala Val Ser Leu Leu Lys Glu Lys Leu Phe Asn Glu Glu Gly Glu
[1094] 35 40 45
[1095] Pro Val Asn Leu Ile Phe Cys Tyr Thr Ile Leu Gln Met Lys Val Ala
[1096] 50 55 60
[1097] Glu Arg Ile Met Ala Gln His Pro Gly Glu Arg Phe Tyr Val Val Leu
[1098] 65 70 75 80
[1099] Met Ser Glu Asn Arg Asn Glu Lys Tyr Asp Tyr Tyr Phe Asn Gln Ile
[1100] 85 90 95
[1101] Lys Asp Lys Ala Glu Arg Ala Tyr Phe Phe His Leu Pro Tyr Gly Leu
[1102] 100 105 110
[1103] Asn Lys Ser Phe Asn Phe Ile Pro Thr Met Ala Glu Leu Lys Val Lys
[1104] 115 120 125
[1105] Ser Met Leu Leu Pro Lys Val Lys Arg Ile Tyr Leu Ala Ser Leu Glu
[1106] 130 135 140
[1107] Lys Val Ser Ile Ala Ala Phe Leu Ser Thr Tyr Pro Asp Ala Glu Ile
[1108] 145 150 155 160
[1109] Lys Thr Phe Asp Asp Gly Thr Gly Asn Leu Ile Gln Ser Ser Ser Tyr
[1110] 165 170 175
[1111] Leu Gly Asp Glu Phe Ser Val Asn Gly Thr Ile Lys Arg Asn Phe Ala
[1112] 180 185 190
[1113] Arg Met Met Ile Gly Asp Trp Ser Ile Ala Lys Thr Arg Asn Ala Ser
[1114] 195 200 205
[1115] Asp Glu His Tyr Thr Ile Phe Lys Gly Leu Lys Asn Ile Met Asp Asp
[1116] 210 215 220
[1117] Gly Arg Arg Lys Met Thr Tyr Leu Pro Leu Phe Asp Ala Ser Glu Leu
[1118] 225 230 235 240
[1119] Lys Thr Gly Asp Glu Thr Gly Gly Thr Val Arg Ile Leu Leu Gly Ser
[1120] 245 250 255
[1121] Pro Asp Lys Glu Met Lys Glu Ile Ser Glu Lys Ala Ala Lys Asn Phe
[1122] 260 265 270
[1123] Lys Ile Gln Tyr Val Ala Pro His Pro Arg Gln Thr Tyr Gly Leu Ser
[1124] 275 280 285
[1125] Gly Val Thr Thr Leu Asn Ser Pro Tyr Val Ile Glu Asp Tyr Ile Leu
[1126] 290 295 300
[1127] Arg Glu Ile Lys Lys Asn Pro His Thr Arg Tyr Glu Ile Tyr Thr Phe
[1128] 305 310 315 320
[1129] Phe Ser Gly Ala Ala Leu Thr Met Lys Asp Phe Pro Asn Val His Val
[1130] 325 330 335
[1131] Tyr Ala Leu Lys Pro Ala Ser Leu Pro Glu Asp Tyr Trp Leu Lys Pro
[1132] 340 345 350
[1133] Val Tyr Ala Leu Phe Thr Gln Ser Gly Ile Pro Ile Leu Thr Phe Asp

[1134] 355 360 365
[1135] Asp Lys Asn
[1136] 370
[1137] <210> 39
[1138] <211> 283
[1139] <212> PRT
[1140] <213> 多杀性巴氏杆菌(Pasteurella multocida)
[1141] <400> 39
[1142] Met Asp Lys Phe Ala Glu His Glu Ile Pro Lys Ala Val Ile Val Ala
[1143] 1 5 10 15
[1144] Gly Asn Gly Glu Ser Leu Ser Gln Ile Asp Tyr Arg Leu Leu Pro Lys
[1145] 20 25 30
[1146] Asn Tyr Asp Val Phe Arg Cys Asn Gln Phe Tyr Phe Glu Glu Arg Tyr
[1147] 35 40 45
[1148] Phe Leu Gly Asn Lys Ile Lys Ala Val Phe Phe Thr Pro Gly Val Phe
[1149] 50 55 60
[1150] Leu Glu Gln Tyr Tyr Thr Leu Tyr His Leu Lys Arg Asn Asn Glu Tyr
[1151] 65 70 75 80
[1152] Phe Val Asp Asn Val Ile Leu Ser Ser Phe Asn His Pro Thr Val Asp
[1153] 85 90 95
[1154] Leu Glu Lys Ser Gln Lys Ile Gln Ala Leu Phe Ile Asp Val Ile Asn
[1155] 100 105 110
[1156] Gly Tyr Glu Lys Tyr Leu Ser Lys Leu Thr Ala Phe Asp Val Tyr Leu
[1157] 115 120 125
[1158] Arg Tyr Lys Glu Leu Tyr Glu Asn Gln Arg Ile Thr Ser Gly Val Tyr
[1159] 130 135 140
[1160] Met Cys Ala Val Ala Ile Ala Met Gly Tyr Thr Asp Ile Tyr Leu Thr
[1161] 145 150 155 160
[1162] Gly Ile Asp Phe Tyr Gln Ala Ser Glu Glu Asn Tyr Ala Phe Asp Asn
[1163] 165 170 175
[1164] Lys Lys Pro Asn Ile Ile Arg Leu Leu Pro Asp Phe Arg Lys Glu Lys
[1165] 180 185 190
[1166] Thr Leu Phe Ser Tyr His Ser Lys Asp Ile Asp Leu Glu Ala Leu Ser
[1167] 195 200 205
[1168] Phe Leu Gln Gln His Tyr His Val Asn Phe Tyr Ser Ile Ser Pro Met
[1169] 210 215 220
[1170] Ser Pro Leu Ser Lys His Phe Pro Ile Pro Thr Val Glu Asp Asp Cys
[1171] 225 230 235 240
[1172] Glu Thr Thr Phe Val Ala Pro Leu Lys Glu Asn Tyr Ile Asn Asp Ile
[1173] 245 250 255
[1174] Leu Leu Pro Pro His Phe Val Tyr Glu Lys Leu Gly Val Asp Lys Leu
[1175] 260 265 270

[1176] Ala Ala Ala Leu Glu His His His His His His
[1177] 275 280
[1178] <210> 40
[1179] <211> 385
[1180] <212> PRT
[1181] <213> 达可马巴斯德氏菌(Pasteurella dagmatis)
[1182] <400> 40
[1183] Met Thr Ile Tyr Leu Asp Pro Ala Ser Leu Pro Thr Leu Asn Gln Leu
[1184] 1 5 10 15
[1185] Met His Phe Thr Lys Glu Ser Glu Asp Lys Glu Thr Ala Arg Ile Phe
[1186] 20 25 30
[1187] Gly Phe Ser Arg Phe Lys Leu Pro Glu Lys Ile Thr Glu Gln Tyr Asn
[1188] 35 40 45
[1189] Asn Ile His Phe Val Glu Ile Lys Asn Asn Arg Pro Thr Glu Asp Ile
[1190] 50 55 60
[1191] Phe Thr Ile Leu Asp Gln Tyr Pro Glu Lys Leu Glu Leu Asp Leu His
[1192] 65 70 75 80
[1193] Leu Asn Ile Ala His Ser Ile Gln Leu Phe His Pro Ile Leu Gln Tyr
[1194] 85 90 95
[1195] Arg Phe Lys His Pro Asp Arg Ile Ser Ile Lys Ser Leu Asn Leu Tyr
[1196] 100 105 110
[1197] Asp Asp Gly Thr Met Glu Tyr Val Asp Leu Glu Lys Glu Glu Asn Lys
[1198] 115 120 125
[1199] Asp Ile Lys Ser Ala Ile Lys Lys Ala Glu Lys Gln Leu Ser Asp Tyr
[1200] 130 135 140
[1201] Leu Leu Thr Gly Lys Ile Asn Phe Asp Asn Pro Thr Leu Ala Arg Tyr
[1202] 145 150 155 160
[1203] Val Trp Gln Ser Gln Tyr Pro Val Lys Tyr His Phe Leu Ser Thr Glu
[1204] 165 170 175
[1205] Tyr Phe Glu Lys Ala Glu Phe Leu Gln Pro Leu Lys Thr Tyr Leu Ala
[1206] 180 185 190
[1207] Gly Lys Tyr Gln Lys Met Asp Trp Ser Ala Tyr Glu Lys Leu Ser Pro
[1208] 195 200 205
[1209] Glu Gln Gln Thr Phe Tyr Leu Lys Leu Val Gly Phe Ser Asp Glu Thr
[1210] 210 215 220
[1211] Lys Gln Leu Phe His Thr Glu Gln Thr Lys Phe Ile Phe Thr Gly Thr
[1212] 225 230 235 240
[1213] Thr Thr Trp Glu Gly Asn Thr Asp Ile Arg Glu Tyr Tyr Ala Lys Gln
[1214] 245 250 255
[1215] Gln Leu Asn Leu Leu Lys His Phe Thr His Ser Glu Gly Asp Leu Phe
[1216] 260 265 270
[1217] Ile Gly Asp Gln Tyr Lys Ile Tyr Phe Lys Gly His Pro Arg Gly Gly

[1218] 275 280 285
[1219] Asp Ile Asn Asp Tyr Ile Leu Lys His Ala Lys Asp Ile Thr Asn Ile
[1220] 290 295 300
[1221] Pro Ala Asn Ile Ser Phe Glu Ile Leu Met Met Thr Gly Leu Leu Pro
[1222] 305 310 315 320
[1223] Asp Lys Val Gly Gly Val Ala Ser Ser Leu Tyr Phe Ser Leu Pro Lys
[1224] 325 330 335
[1225] Glu Lys Ile Ser His Ile Ile Phe Thr Ser Asn Lys Lys Ile Lys Asn
[1226] 340 345 350
[1227] Lys Glu Asp Ala Leu Asn Asp Pro Tyr Val Arg Val Met Leu Arg Leu
[1228] 355 360 365
[1229] Gly Met Ile Asp Lys Ser Gln Ile Ile Phe Trp Asp Ser Leu Lys Gln
[1230] 370 375 380
[1231] Leu
[1232] 385
[1233] <210> 41
[1234] <211> 390
[1235] <212> PRT
[1236] <213> 明亮发光杆菌(Photobacterium phosphoreum)
[1237] <400> 41
[1238] Met Gly Cys Asn Ser Asp Ser Lys His Asn Asn Ser Asp Gly Asn Ile
[1239] 1 5 10 15
[1240] Thr Lys Asn Lys Thr Ile Glu Val Tyr Val Asp Arg Ala Thr Leu Pro
[1241] 20 25 30
[1242] Thr Ile Gln Gln Met Thr Gln Ile Ile Asn Glu Asn Ser Asn Asn Lys
[1243] 35 40 45
[1244] Lys Leu Ile Ser Trp Ser Arg Tyr Pro Ile Asn Asp Glu Thr Leu Leu
[1245] 50 55 60
[1246] Glu Ser Ile Asn Gly Ser Phe Phe Lys Asn Arg Pro Glu Leu Ile Lys
[1247] 65 70 75 80
[1248] Ser Leu Asp Ser Met Ile Leu Thr Asn Glu Ile Lys Lys Val Ile Ile
[1249] 85 90 95
[1250] Asn Gly Asn Thr Leu Trp Ala Val Asp Val Val Asn Ile Ile Lys Ser
[1251] 100 105 110
[1252] Ile Glu Ala Leu Gly Lys Lys Thr Glu Ile Glu Leu Asn Phe Tyr Asp
[1253] 115 120 125
[1254] Asp Gly Ser Ala Glu Tyr Val Arg Leu Tyr Asp Phe Ser Arg Leu Pro
[1255] 130 135 140
[1256] Glu Ser Glu Gln Glu Tyr Lys Ile Ser Leu Ser Lys Asp Asn Ile Gln
[1257] 145 150 155 160
[1258] Ser Ser Ile Asn Gly Thr Gln Pro Phe Asp Asn Ser Ile Glu Asn Ile
[1259] 165 170 175

[1260] Tyr Gly Phe Ser Gln Leu Tyr Pro Thr Thr Tyr His Met Leu Arg Ala
[1261] 180 185 190
[1262] Asp Ile Phe Glu Thr Asn Leu Pro Leu Thr Ser Leu Lys Arg Val Ile
[1263] 195 200 205
[1264] Ser Asn Asn Ile Lys Gln Met Lys Trp Asp Tyr Phe Thr Thr Phe Asn
[1265] 210 215 220
[1266] Ser Gln Gln Lys Asn Lys Phe Tyr Asn Phe Thr Gly Phe Asn Pro Glu
[1267] 225 230 235 240
[1268] Lys Ile Lys Glu Gln Tyr Lys Ala Ser Pro His Glu Asn Phe Ile Phe
[1269] 245 250 255
[1270] Ile Gly Thr Asn Ser Gly Thr Ala Thr Ala Glu Gln Gln Ile Asp Ile
[1271] 260 265 270
[1272] Leu Thr Glu Ala Lys Lys Pro Asp Ser Pro Ile Ile Thr Asn Ser Ile
[1273] 275 280 285
[1274] Gln Gly Leu Asp Leu Phe Phe Lys Gly His Pro Ser Ala Thr Tyr Asn
[1275] 290 295 300
[1276] Gln Gln Ile Ile Asp Ala His Asn Met Ile Glu Ile Tyr Asn Lys Ile
[1277] 305 310 315 320
[1278] Pro Phe Glu Ala Leu Ile Met Thr Asp Ala Leu Pro Asp Ala Val Gly
[1279] 325 330 335
[1280] Gly Met Gly Ser Ser Val Phe Phe Ser Leu Pro Asn Thr Val Glu Asn
[1281] 340 345 350
[1282] Lys Phe Ile Phe Tyr Lys Ser Asp Thr Asp Ile Glu Asn Asn Ala Leu
[1283] 355 360 365
[1284] Ile Gln Val Met Ile Glu Leu Asn Ile Val Asn Arg Asn Asp Val Lys
[1285] 370 375 380
[1286] Leu Ile Ser Asp Leu Gln
[1287] 385 390
[1288] <210> 42
[1289] <211> 417
[1290] <212> PRT
[1291] <213> 副鸡禽杆菌(Avibacterium paragallinarum)
[1292] <400> 42
[1293] Met Arg Lys Ile Ile Thr Phe Phe Ser Leu Phe Phe Ser Ile Ser Ala
[1294] 1 5 10 15
[1295] Trp Cys Gln Lys Met Glu Ile Tyr Leu Asp Tyr Ala Ser Leu Pro Ser
[1296] 20 25 30
[1297] Leu Asn Met Ile Leu Asn Leu Val Glu Asn Lys Asn Asn Glu Lys Val
[1298] 35 40 45
[1299] Glu Arg Ile Ile Gly Phe Glu Arg Phe Asp Phe Asn Lys Glu Ile Leu
[1300] 50 55 60
[1301] Asn Ser Phe Ser Lys Glu Arg Ile Glu Phe Ser Lys Val Ser Ile Leu

[1302] 65 70 75 80
[1303] Asp Ile Lys Glu Phe Ser Asp Lys Leu Tyr Leu Asn Ile Glu Lys Ser
[1304] 85 90 95
[1305] Asp Thr Pro Val Asp Leu Ile Ile His Thr Asn Leu Asp His Ser Val
[1306] 100 105 110
[1307] Arg Ser Leu Leu Ser Ile Phe Lys Thr Leu Ser Pro Leu Phe His Lys
[1308] 115 120 125
[1309] Ile Asn Ile Glu Lys Leu Tyr Leu Tyr Asp Asp Gly Ser Gly Asn Tyr
[1310] 130 135 140
[1311] Val Asp Leu Tyr Gln His Arg Gln Glu Asn Ile Ser Ala Ile Leu Ile
[1312] 145 150 155 160
[1313] Glu Ala Gln Lys Lys Leu Lys Asp Ala Leu Glu Asn Arg Glu Thr Asp
[1314] 165 170 175
[1315] Thr Asp Lys Leu His Ser Leu Thr Arg Tyr Thr Trp His Lys Ile Phe
[1316] 180 185 190
[1317] Pro Thr Glu Tyr Ile Leu Leu Arg Pro Asp Tyr Leu Asp Ile Asp Glu
[1318] 195 200 205
[1319] Lys Met Gln Pro Leu Lys His Phe Leu Ser Asp Thr Ile Val Ser Met
[1320] 210 215 220
[1321] Asp Leu Ser Arg Phe Ser His Phe Ser Lys Asn Gln Lys Glu Leu Phe
[1322] 225 230 235 240
[1323] Leu Lys Ile Thr His Phe Asp Gln Asn Ile Phe Asn Glu Leu Asn Ile
[1324] 245 250 255
[1325] Gly Thr Lys Asn Lys Glu Tyr Lys Thr Phe Ile Phe Thr Gly Thr Thr
[1326] 260 265 270
[1327] Thr Trp Glu Lys Asp Lys Lys Lys Arg Leu Asn Asn Ala Lys Leu Gln
[1328] 275 280 285
[1329] Thr Glu Ile Leu Glu Ser Phe Ile Lys Pro Asn Gly Lys Phe Tyr Leu
[1330] 290 295 300
[1331] Gly Asn Asp Ile Lys Ile Phe Phe Lys Gly His Pro Lys Gly Asp Asp
[1332] 305 310 315 320
[1333] Ile Asn Asp Tyr Ile Ile Arg Lys Thr Gly Ala Glu Lys Ile Pro Ala
[1334] 325 330 335
[1335] Asn Ile Pro Phe Glu Val Leu Met Met Thr Asn Ser Leu Pro Asp Tyr
[1336] 340 345 350
[1337] Val Gly Gly Ile Met Ser Thr Val Tyr Phe Ser Leu Pro Pro Lys Asn
[1338] 355 360 365
[1339] Ile Asp Lys Val Val Phe Leu Gly Ser Glu Lys Ile Lys Asn Glu Asn
[1340] 370 375 380
[1341] Asp Ala Lys Ser Gln Thr Leu Ser Lys Leu Met Leu Met Leu Asn Val
[1342] 385 390 395 400
[1343] Ile Thr Pro Glu Gln Ile Phe Phe Glu Glu Met Pro Asn Pro Ile Asn

[1344] 405 410 415

[1345] Phe

[1346] <210> 43

[1347] <211> 430

[1348] <212> PRT

[1349] <213> 空肠弯曲杆菌(Campylobacter jejuni)

[1350] <400> 43

[1351] Met Thr Arg Thr Arg Met Glu Asn Glu Leu Ile Val Ser Lys Asn Met

[1352] 1 5 10 15

[1353] Gln Asn Ile Ile Ile Ala Gly Asn Gly Pro Ser Leu Lys Asn Ile Asn

[1354] 20 25 30

[1355] Tyr Lys Arg Leu Pro Arg Glu Tyr Asp Val Phe Arg Cys Asn Gln Phe

[1356] 35 40 45

[1357] Tyr Phe Glu Asp Lys Tyr Tyr Leu Gly Lys Lys Ile Lys Ala Val Phe

[1358] 50 55 60

[1359] Phe Asn Pro Gly Val Phe Leu Gln Gln Tyr His Thr Ala Lys Gln Leu

[1360] 65 70 75 80

[1361] Ile Leu Lys Asn Glu Tyr Glu Ile Lys Asn Ile Phe Cys Ser Thr Phe

[1362] 85 90 95

[1363] Asn Leu Pro Phe Ile Glu Ser Asn Asp Phe Leu His Gln Phe Tyr Asn

[1364] 100 105 110

[1365] Phe Phe Pro Asp Ala Lys Leu Gly Tyr Glu Val Ile Glu Asn Leu Lys

[1366] 115 120 125

[1367] Glu Phe Tyr Ala Tyr Ile Lys Tyr Asn Glu Ile Tyr Phe Asn Lys Arg

[1368] 130 135 140

[1369] Ile Thr Ser Gly Val Tyr Met Cys Ala Ile Ala Ile Ala Leu Gly Tyr

[1370] 145 150 155 160

[1371] Lys Thr Ile Tyr Leu Cys Gly Ile Asp Phe Tyr Glu Gly Asp Val Ile

[1372] 165 170 175

[1373] Tyr Pro Phe Glu Ala Met Ser Thr Asn Ile Lys Thr Ile Phe Pro Gly

[1374] 180 185 190

[1375] Ile Lys Asp Phe Lys Pro Ser Asn Cys His Ser Lys Glu Tyr Asp Ile

[1376] 195 200 205

[1377] Glu Ala Leu Lys Leu Leu Lys Ser Ile Tyr Lys Val Asn Ile Tyr Ala

[1378] 210 215 220

[1379] Leu Cys Asp Asp Ser Ile Leu Ala Asn His Phe Pro Leu Ser Ile Asn

[1380] 225 230 235 240

[1381] Ile Asn Asn Asn Phe Thr Leu Glu Asn Lys His Asn Asn Ser Ile Asn

[1382] 245 250 255

[1383] Asp Ile Leu Leu Thr Asp Asn Thr Pro Gly Val Ser Phe Tyr Lys Asn

[1384] 260 265 270

[1385] Gln Leu Lys Ala Asp Asn Lys Ile Met Leu Asn Phe Tyr Asn Ile Leu

[1386] 275 280 285
[1387] His Ser Lys Asp Asn Leu Ile Lys Phe Leu Asn Lys Glu Ile Ala Val
[1388] 290 295 300
[1389] Leu Lys Lys Gln Thr Thr Gln Arg Ala Lys Ala Arg Ile Gln Asn His
[1390] 305 310 315 320
[1391] Leu Ser Tyr Lys Leu Gly Gln Ala Leu Ile Ile Asn Ser Lys Ser Val
[1392] 325 330 335
[1393] Leu Gly Phe Leu Ser Leu Pro Phe Ile Ile Leu Ser Ile Val Ile Ser
[1394] 340 345 350
[1395] His Lys Gln Glu Gln Lys Ala Tyr Lys Phe Lys Val Lys Lys Asn Pro
[1396] 355 360 365
[1397] Asn Leu Ala Leu Pro Pro Leu Glu Thr Tyr Pro Asp Tyr Asn Glu Ala
[1398] 370 375 380
[1399] Leu Lys Glu Lys Glu Cys Phe Thr Tyr Lys Leu Gly Glu Glu Phe Ile
[1400] 385 390 395 400
[1401] Lys Ala Gly Lys Asn Trp Tyr Gly Glu Gly Tyr Ile Lys Phe Ile Phe
[1402] 405 410 415
[1403] Lys Asp Val Pro Arg Leu Lys Arg Glu Phe Glu Lys Gly Glu
[1404] 420 425 430
[1405] <210> 44
[1406] <211> 395
[1407] <212> PRT
[1408] <213> 蓖麻毒素螺杆菌(Heliobacter acinonychis)
[1409] <400> 44
[1410] Met Asn Lys Lys Pro Leu Ile Ile Ala Gly Asn Gly Pro Ser Ile Lys
[1411] 1 5 10 15
[1412] Asp Leu Asp Tyr Ala Leu Phe Pro Lys Asp Phe Asp Val Phe Arg Cys
[1413] 20 25 30
[1414] Asn Gln Phe Tyr Phe Glu Asp Lys Tyr Tyr Leu Gly Arg Glu Ile Lys
[1415] 35 40 45
[1416] Gly Val Phe Phe Asn Ala His Val Phe Asp Leu Gln Met Lys Ile Thr
[1417] 50 55 60
[1418] Lys Ala Ile Val Lys Asn Gly Glu Tyr His Pro Asp His Ile Tyr Cys
[1419] 65 70 75 80
[1420] Thr His Val Glu Pro Tyr Gly Tyr Val Asn Gly Asn Gln Gln Leu Met
[1421] 85 90 95
[1422] Gln Glu Tyr Leu Glu Lys His Phe Val Gly Val Arg Ser Thr Tyr Ala
[1423] 100 105 110
[1424] Tyr Leu Lys Asp Leu Glu Pro Phe Phe Ile Leu His Ser Lys Tyr Arg
[1425] 115 120 125
[1426] Asn Phe Tyr Asp Gln His Phe Thr Thr Gly Ile Met Met Leu Leu Val
[1427] 130 135 140

[1428] Ala Ile Gln Leu Gly Tyr Lys Glu Ile Tyr Leu Cys Gly Ile Asp Phe
[1429] 145 150 155 160
[1430] Tyr Glu Asn Gly Phe Gly His Phe Tyr Glu Asn Gln Gly Gly Phe Phe
[1431] 165 170 175
[1432] Glu Glu Asp Ser Asp Pro Met His Asp Lys Asn Ile Asp Ile Gln Ala
[1433] 180 185 190
[1434] Leu Glu Leu Ala Lys Lys Tyr Ala Lys Ile Tyr Ala Leu Val Pro Asn
[1435] 195 200 205
[1436] Ser Ala Leu Val Lys Met Ile Pro Leu Ser Ser Gln Lys Gly Val Leu
[1437] 210 215 220
[1438] Glu Lys Val Lys Asp Arg Ile Gly Leu Gly Glu Phe Lys Arg Glu Lys
[1439] 225 230 235 240
[1440] Phe Gly Gln Lys Glu Leu Glu Arg Gln Lys Glu Leu Glu Arg Gln Lys
[1441] 245 250 255
[1442] Glu Leu Glu Arg Gln Lys Glu Leu Glu Arg Gln Lys Glu Leu Glu Arg
[1443] 260 265 270
[1444] Gln Lys Glu Leu Glu Arg Gln Lys Glu Leu Glu Arg Gln Lys Glu Leu
[1445] 275 280 285
[1446] Glu Arg Gln Lys Glu Leu Glu Arg Gln Lys Glu Leu Glu Arg Gln Lys
[1447] 290 295 300
[1448] Glu Leu Glu Arg Gln Lys Glu Leu Glu Arg Gln Lys Glu Leu Glu Arg
[1449] 305 310 315 320
[1450] Gln Lys Glu Leu Glu Arg Gln Lys Glu Leu Glu Arg Gln Lys Glu Leu
[1451] 325 330 335
[1452] Glu Leu Glu Arg Ser Leu Lys Ala Arg Leu Lys Ala Val Leu Ala Ser
[1453] 340 345 350
[1454] Lys Gly Ile Arg Gly Asp Asn Leu Ile Ile Val Ser Leu Lys Asp Thr
[1455] 355 360 365
[1456] Tyr Arg Leu Phe Lys Gly Gly Phe Ala Leu Leu Leu Asp Leu Lys Ala
[1457] 370 375 380
[1458] Leu Lys Ser Ile Ile Lys Ala Phe Leu Lys Arg
[1459] 385 390 395
[1460] <210> 45
[1461] <211> 260
[1462] <212> PRT
[1463] <213> 空肠弯曲杆菌(Campylobacter jejuni)
[1464] <400> 45
[1465] Met Gly Lys Lys Val Ile Ile Ala Gly Asn Gly Pro Ser Leu Lys Glu
[1466] 1 5 10 15
[1467] Ile Asp Tyr Ser Arg Leu Pro Asn Asp Phe Asp Val Phe Arg Cys Asn
[1468] 20 25 30
[1469] Gln Phe Tyr Phe Glu Asp Lys Tyr Tyr Leu Gly Lys Lys Cys Lys Ala

[1470] 35 40 45
[1471] Val Phe Tyr Asn Pro Ile Leu Phe Phe Glu Gln Tyr Tyr Thr Leu Lys
[1472] 50 55 60
[1473] His Leu Ile Gln Asn Gln Glu Tyr Glu Thr Glu Leu Ile Met Cys Ser
[1474] 65 70 75 80
[1475] Asn Tyr Asn Gln Ala His Leu Glu Asn Glu Asn Phe Val Lys Thr Phe
[1476] 85 90 95
[1477] Tyr Asp Tyr Phe Pro Asp Ala His Leu Gly Tyr Asp Phe Phe Lys Gln
[1478] 100 105 110
[1479] Leu Lys Asp Phe Asn Ala Tyr Phe Lys Phe His Glu Ile Tyr Phe Asn
[1480] 115 120 125
[1481] Gln Arg Ile Thr Ser Gly Val Tyr Met Cys Ala Val Ala Ile Ala Leu
[1482] 130 135 140
[1483] Gly Tyr Lys Glu Ile Tyr Leu Ser Gly Ile Asp Phe Tyr Gln Asn Gly
[1484] 145 150 155 160
[1485] Ser Ser Tyr Ala Phe Asp Thr Lys Gln Lys Asn Leu Leu Lys Leu Ala
[1486] 165 170 175
[1487] Pro Asn Phe Lys Asn Asp Asn Ser His Tyr Ile Gly His Ser Lys Asn
[1488] 180 185 190
[1489] Thr Asp Ile Lys Ala Leu Glu Phe Leu Glu Lys Thr Tyr Lys Ile Lys
[1490] 195 200 205
[1491] Leu Tyr Cys Leu Cys Pro Asn Ser Leu Leu Ala Asn Phe Ile Glu Leu
[1492] 210 215 220
[1493] Ala Pro Asn Leu Asn Ser Asn Phe Ile Ile Gln Glu Lys Asn Asn Tyr
[1494] 225 230 235 240
[1495] Thr Lys Asp Ile Leu Ile Pro Ser Ser Glu Ala Tyr Gly Lys Phe Ser
[1496] 245 250 255
[1497] Lys Asn Ile Asn
[1498] 260
[1499] <210> 46
[1500] <211> 298
[1501] <212> PRT
[1502] <213> 肠链球菌(Streptococcus entericus)
[1503] <400> 46
[1504] Met Lys Lys Val Tyr Phe Cys His Thr Val Tyr His Leu Leu Ile Thr
[1505] 1 5 10 15
[1506] Leu Cys Lys Ile Ser Val Glu Glu Gln Val Glu Ile Ile Val Phe Asp
[1507] 20 25 30
[1508] Thr Val Ser Asn His Glu Leu Ile Val Gln Lys Ile Arg Asp Val Phe
[1509] 35 40 45
[1510] Val Asn Thr Thr Val Leu Phe Ala Glu Gln Asn Thr Asp Phe Ser Ile
[1511] 50 55 60

[1512] Leu Glu Ile Asp Arg Ala Thr Asp Ile Tyr Val Phe Asn Asp Trp Thr
[1513] 65 70 75 80
[1514] Pro Ile Gly Ala Tyr Leu Arg Lys Asn Lys Leu Phe Tyr His Leu Ile
[1515] 85 90 95
[1516] Glu Asp Gly Tyr Asn Tyr His Glu Tyr Asn Val Tyr Ala Asn Ala Leu
[1517] 100 105 110
[1518] Thr Met Lys Arg Arg Leu Leu Asn Phe Val Leu Arg Arg Glu Glu Pro
[1519] 115 120 125
[1520] Ser Gly Phe Ser Arg Tyr Val Arg Ser Ile Glu Val Asn Arg Val Lys
[1521] 130 135 140
[1522] Tyr Leu Pro Asn Asp Cys Arg Lys Ser Lys Trp Val Glu Lys Pro Arg
[1523] 145 150 155 160
[1524] Ser Ala Leu Phe Glu Asn Leu Val Pro Glu His Lys Gln Lys Ile Ile
[1525] 165 170 175
[1526] Thr Ile Phe Gly Leu Glu Asn Tyr Gln Asp Ser Leu Arg Gly Val Leu
[1527] 180 185 190
[1528] Val Leu Thr Gln Pro Leu Val Gln Asp Tyr Trp Asp Arg Asp Ile Thr
[1529] 195 200 205
[1530] Thr Glu Glu Glu Gln Leu Glu Phe Tyr Arg Gln Ile Val Glu Ser Tyr
[1531] 210 215 220
[1532] Gly Glu Gly Glu Gln Val Phe Phe Lys Ile His Pro Arg Asp Lys Val
[1533] 225 230 235 240
[1534] Asp Tyr Ser Ser Leu Thr Asn Val Ile Phe Leu Lys Lys Asn Val Pro
[1535] 245 250 255
[1536] Met Glu Val Tyr Glu Leu Ile Ala Asp Cys His Phe Thr Lys Gly Ile
[1537] 260 265 270
[1538] Thr His Ser Ser Thr Ala Leu Asp Phe Leu Ser Cys Val Asp Lys Lys
[1539] 275 280 285
[1540] Ile Thr Leu Lys Gln Met Lys Ala Asn Ser
[1541] 290 295
[1542] <210> 47
[1543] <211> 295
[1544] <212> PRT
[1545] <213> 杜克雷嗜血杆菌(Haemophilus ducreyi)
[1546] <400> 47
[1547] Met Lys Glu Ile Ala Ile Ile Ser Asn Gln Arg Met Phe Phe Leu Tyr
[1548] 1 5 10 15
[1549] Cys Leu Leu Thr Asn Lys Asn Val Glu Asp Val Phe Phe Ile Phe Glu
[1550] 20 25 30
[1551] Lys Gly Ala Met Pro Asn Asn Leu Thr Ser Ile Ser His Phe Ile Val
[1552] 35 40 45
[1553] Leu Asp His Ser Lys Ser Glu Cys Tyr Asp Phe Phe Tyr Phe Asn Phe

[1554] 50 55 60

[1555] Ile Ser Cys Lys Tyr Arg Leu Arg Gly Leu Asp Val Tyr Gly Ala Asp

[1556] 65 70 75 80

[1557] His Ile Lys Gly Ala Lys Phe Phe Leu Glu Arg His Arg Phe Phe Val

[1558] 85 90 95

[1559] Val Glu Asp Gly Met Met Asn Tyr Ser Lys Asn Met Tyr Ala Phe Ser

[1560] 100 105 110

[1561] Leu Phe Arg Thr Arg Asn Pro Val Ile Leu Pro Gly Gly Phe His Pro

[1562] 115 120 125

[1563] Asn Val Lys Thr Ile Phe Leu Thr Lys Asp Asn Pro Ile Pro Asp Gln

[1564] 130 135 140

[1565] Ile Ala His Lys Arg Glu Ile Ile Asn Ile Lys Thr Leu Trp Gln Ala

[1566] 145 150 155 160

[1567] Lys Thr Ala Thr Glu Lys Thr Lys Ile Leu Ser Phe Phe Glu Ile Asp

[1568] 165 170 175

[1569] Met Gln Glu Ile Ser Val Ile Lys Asn Arg Ser Phe Val Leu Tyr Thr

[1570] 180 185 190

[1571] Gln Pro Leu Ser Glu Asp Lys Leu Leu Thr Glu Ala Glu Lys Ile Asp

[1572] 195 200 205

[1573] Ile Tyr Arg Thr Ile Leu Thr Lys Tyr Asn His Ser Gln Thr Val Ile

[1574] 210 215 220

[1575] Lys Pro His Pro Arg Asp Lys Thr Asp Tyr Lys Gln Leu Phe Pro Asp

[1576] 225 230 235 240

[1577] Ala Tyr Val Met Lys Gly Thr Tyr Pro Ser Glu Leu Leu Thr Leu Leu

[1578] 245 250 255

[1579] Gly Val Asn Phe Asn Lys Val Ile Thr Leu Phe Ser Thr Ala Val Phe

[1580] 260 265 270

[1581] Asp Tyr Pro Lys Glu Lys Ile Asp Phe Tyr Gly Thr Ala Val His Pro

[1582] 275 280 285

[1583] Lys Leu Leu Asp Phe Phe Asp

[1584] 290 295

[1585] <210> 48

[1586] <211> 488

[1587] <212> PRT

[1588] <213> 别样杆菌属种(Alistipes sp.)

[1589] <400> 48

[1590] Met Ala Leu Leu Ser Gly Thr Ala Ala Cys Ser Asp Asp Glu Val Ser

[1591] 1 5 10 15

[1592] Gln Asn Leu Ile Val Ile Asn Gly Gly Glu His Phe Leu Ser Leu Asp

[1593] 20 25 30

[1594] Gly Leu Ala Arg Ala Gly Lys Ile Ser Val Leu Ala Pro Ala Pro Trp

[1595] 35 40 45

[1596] Arg Val Thr Lys Ala Ala Gly Asp Thr Trp Phe Arg Leu Ser Ala Thr
[1597] 50 55 60
[1598] Glu Gly Pro Ala Gly Tyr Ser Glu Val Glu Leu Ser Leu Asp Glu Asn
[1599] 65 70 75 80
[1600] Pro Gly Ala Ala Arg Ser Ala Gln Leu Ala Phe Ala Cys Gly Asp Ala
[1601] 85 90 95
[1602] Ile Val Pro Phe Arg Leu Ser Gln Gly Ala Leu Ser Ala Gly Tyr Asp
[1603] 100 105 110
[1604] Ser Pro Asp Tyr Tyr Phe Tyr Val Thr Phe Gly Thr Met Pro Thr Leu
[1605] 115 120 125
[1606] Tyr Ala Gly Ile His Leu Leu Ser His Asp Lys Pro Gly Tyr Val Phe
[1607] 130 135 140
[1608] Tyr Ser Arg Ser Lys Thr Phe Asp Pro Ala Glu Phe Pro Ala Arg Ala
[1609] 145 150 155 160
[1610] Glu Val Thr Thr Ala Ala Asp Arg Thr Ala Asp Ala Thr Gln Ala Glu
[1611] 165 170 175
[1612] Met Glu Ala Met Ala Arg Glu Met Lys Arg Arg Ile Leu Glu Ile Asn
[1613] 180 185 190
[1614] Ser Ala Asp Pro Thr Ala Val Phe Gly Leu Tyr Val Asp Asp Leu Arg
[1615] 195 200 205
[1616] Cys Arg Ile Gly Tyr Asp Trp Phe Val Ala Gln Gly Ile Asp Ser Ala
[1617] 210 215 220
[1618] Arg Val Lys Val Ser Met Leu Ser Asp Gly Thr Gly Thr Tyr Asn Asn
[1619] 225 230 235 240
[1620] Phe Tyr Asn Tyr Phe Gly Asp Ala Ala Thr Ala Glu Gln Asn Trp Glu
[1621] 245 250 255
[1622] Ser Tyr Ala Ser Glu Val Glu Ala Leu Asp Trp Asn His Gly Gly Arg
[1623] 260 265 270
[1624] Tyr Pro Glu Thr Arg Ser Leu Pro Glu Phe Glu Ser Tyr Thr Trp Pro
[1625] 275 280 285
[1626] Tyr Tyr Leu Ser Thr Arg Pro Asp Tyr Arg Leu Val Val Gln Asp Gly
[1627] 290 295 300
[1628] Ser Leu Leu Glu Ser Ser Cys Pro Phe Ile Thr Glu Lys Leu Gly Glu
[1629] 305 310 315 320
[1630] Met Glu Ile Glu Ser Ile Gln Pro Tyr Glu Met Leu Ser Ala Leu Pro
[1631] 325 330 335
[1632] Glu Ser Ser Arg Lys Arg Phe Tyr Asp Met Ala Gly Phe Asp Tyr Asp
[1633] 340 345 350
[1634] Lys Phe Ala Ala Leu Phe Asp Ala Ser Pro Lys Lys Asn Leu Ile Ile
[1635] 355 360 365
[1636] Ile Gly Thr Ser His Ala Asp Asp Ala Ser Ala Arg Leu Gln Arg Asp
[1637] 370 375 380

[1638] Tyr Val Ala Arg Ile Met Glu Gln Tyr Gly Ala Gln Tyr Asp Val Phe
[1639] 385 390 395 400
[1640] Phe Lys Pro His Pro Ala Asp Thr Thr Ser Ala Gly Tyr Glu Thr Glu
[1641] 405 410 415
[1642] Phe Pro Gly Leu Thr Leu Leu Pro Gly Gln Met Pro Phe Glu Ile Phe
[1643] 420 425 430
[1644] Val Trp Ser Leu Ile Asp Arg Val Asp Met Ile Gly Gly Tyr Pro Ser
[1645] 435 440 445
[1646] Thr Val Phe Leu Thr Val Pro Val Asp Lys Val Arg Phe Ile Phe Ala
[1647] 450 455 460
[1648] Ala Asp Ala Ala Ser Leu Val Arg Pro Leu Asn Ile Leu Phe Arg Asp
[1649] 465 470 475 480
[1650] Ala Thr Asp Val Glu Trp Met Gln
[1651] 485
[1652] <210> 49
[1653] <211> 291
[1654] <212> PRT
[1655] <213> 空肠弯曲杆菌(Campylobacter jejuni)
[1656] <400> 49
[1657] Met Lys Lys Val Ile Ile Ala Gly Asn Gly Pro Ser Leu Lys Glu Ile
[1658] 1 5 10 15
[1659] Asp Tyr Ser Arg Leu Pro Asn Asp Phe Asp Val Phe Arg Cys Asn Gln
[1660] 20 25 30
[1661] Phe Tyr Phe Glu Asp Lys Tyr Tyr Leu Gly Lys Lys Cys Lys Ala Val
[1662] 35 40 45
[1663] Phe Tyr Thr Pro Asn Phe Phe Phe Glu Gln Tyr Tyr Thr Leu Lys His
[1664] 50 55 60
[1665] Leu Ile Gln Asn Gln Glu Tyr Glu Thr Glu Leu Ile Met Cys Ser Asn
[1666] 65 70 75 80
[1667] Tyr Asn Gln Ala His Leu Glu Asn Glu Asn Phe Val Lys Thr Phe Tyr
[1668] 85 90 95
[1669] Asp Tyr Phe Pro Asp Ala His Leu Gly Tyr Asp Phe Phe Lys Gln Leu
[1670] 100 105 110
[1671] Lys Glu Phe Asn Ala Tyr Phe Lys Phe His Glu Ile Tyr Phe Asn Gln
[1672] 115 120 125
[1673] Arg Ile Thr Ser Gly Val Tyr Met Cys Ala Val Ala Ile Ala Leu Gly
[1674] 130 135 140
[1675] Tyr Lys Glu Ile Tyr Leu Ser Gly Ile Asp Phe Tyr Gln Asn Gly Ser
[1676] 145 150 155 160
[1677] Ser Tyr Ala Phe Asp Thr Lys Gln Glu Asn Leu Leu Lys Leu Ala Pro
[1678] 165 170 175
[1679] Asp Phe Lys Asn Asp Arg Ser His Tyr Ile Gly His Ser Lys Asn Thr

[1680] 180 185 190
[1681] Asp Ile Lys Ala Leu Glu Phe Leu Glu Lys Thr Tyr Lys Ile Lys Leu
[1682] 195 200 205
[1683] Tyr Cys Leu Cys Pro Asn Ser Leu Leu Ala Asn Phe Ile Glu Leu Ala
[1684] 210 215 220
[1685] Pro Asn Leu Asn Ser Asn Phe Ile Ile Gln Glu Lys Asn Asn Tyr Thr
[1686] 225 230 235 240
[1687] Lys Asp Ile Leu Ile Pro Ser Ser Glu Ala Tyr Gly Lys Phe Ser Lys
[1688] 245 250 255
[1689] Asn Ile Asn Phe Lys Lys Ile Lys Ile Lys Glu Asn Val Tyr Tyr Lys
[1690] 260 265 270
[1691] Leu Ile Lys Asp Leu Leu Arg Leu Pro Ser Asp Ile Lys His Tyr Phe
[1692] 275 280 285
[1693] Lys Gly Lys
[1694] 290
[1695] <210> 50
[1696] <211> 312
[1697] <212> PRT
[1698] <213> 无乳链球菌(Streptococcus agalactiae)
[1699] <400> 50
[1700] Met Thr Asn Arg Lys Ile Tyr Val Cys His Thr Leu Tyr His Leu Leu
[1701] 1 5 10 15
[1702] Ile Cys Leu Tyr Lys Glu Glu Ile Tyr Ser Asn Leu Glu Ile Ile Leu
[1703] 20 25 30
[1704] Ser Ser Ser Ile Pro Asp Val Asp Asn Leu Glu Lys Lys Leu Lys Ser
[1705] 35 40 45
[1706] Lys Thr Ile Asn Ile His Ile Leu Glu Glu Ser Ser Gly Glu Ser Glu
[1707] 50 55 60
[1708] Glu Leu Leu Ser Val Leu Lys Asp Ala Gly Leu Ser Tyr Ser Lys Phe
[1709] 65 70 75 80
[1710] Asp Ser Asn Cys Phe Ile Phe Asn Asp Ala Thr Pro Ile Gly Arg Thr
[1711] 85 90 95
[1712] Leu Ile Lys His Gly Ile Tyr Tyr Asn Leu Ile Glu Asp Gly Leu Asn
[1713] 100 105 110
[1714] Cys Phe Thr Tyr Ser Ile Phe Ser Gln Lys Leu Trp Lys Tyr Tyr Val
[1715] 115 120 125
[1716] Lys Lys Tyr Ile Leu His Lys Ile Gln Pro His Gly Phe Ser Arg Tyr
[1717] 130 135 140
[1718] Cys Leu Gly Ile Glu Val Asn Ser Leu Val Asn Leu Pro Lys Asp Pro
[1719] 145 150 155 160
[1720] Arg Tyr Lys Lys Phe Ile Glu Val Pro Arg Lys Glu Leu Phe Asp Asn
[1721] 165 170 175

[1722] Val Thr Glu Tyr Gln Lys Glu Met Ala Ile Asn Leu Phe Gly Ala Val
[1723] 180 185 190
[1724] Arg Val Ser Ile Lys Ser Pro Ser Val Leu Val Leu Thr Gln Pro Leu
[1725] 195 200 205
[1726] Ser Ile Asp Lys Glu Phe Met Ser Tyr Asn Asn Lys Ile Glu Thr Ser
[1727] 210 215 220
[1728] Glu Glu Gln Phe Asn Phe Tyr Lys Ser Ile Val Asn Glu Tyr Ile Asn
[1729] 225 230 235 240
[1730] Lys Gly Tyr Asn Val Tyr Leu Lys Val His Pro Arg Asp Val Val Asp
[1731] 245 250 255
[1732] Tyr Ser Lys Leu Pro Val Glu Leu Leu Pro Ser Asn Val Pro Met Glu
[1733] 260 265 270
[1734] Ile Ile Glu Leu Met Leu Thr Gly Arg Phe Glu Cys Gly Ile Thr His
[1735] 275 280 285
[1736] Ser Ser Thr Ala Leu Asp Phe Leu Thr Cys Val Asp Lys Lys Ile Thr
[1737] 290 295 300
[1738] Leu Val Asp Leu Lys Asp Ile Lys
[1739] 305 310
[1740] <210> 51
[1741] <211> 410
[1742] <212> PRT
[1743] <213> 山羊海藻百伯史坦菌(Bibersteinia trehalosi)
[1744] <400> 51
[1745] Met Glu Phe Cys Lys Met Ala Thr Thr Gln Lys Ile Cys Val Tyr Leu
[1746] 1 5 10 15
[1747] Asp Tyr Ala Thr Ile Pro Ser Leu Asn Tyr Ile Leu His Phe Ala Gln
[1748] 20 25 30
[1749] His Phe Glu Asp Gln Glu Thr Ile Arg Leu Phe Gly Leu Ser Arg Phe
[1750] 35 40 45
[1751] His Ile Pro Glu Ser Val Ile Gln Arg Tyr Pro Lys Gly Val Val Gln
[1752] 50 55 60
[1753] Phe Tyr Pro Asn Gln Glu Lys Asp Phe Ser Ala Leu Leu Leu Ala Leu
[1754] 65 70 75 80
[1755] Lys Asn Ile Leu Ile Glu Val Lys Gln Gln Gln Arg Lys Cys Glu Ile
[1756] 85 90 95
[1757] Glu Leu His Leu Asn Leu Phe His Tyr Gln Leu Leu Leu Leu Pro Phe
[1758] 100 105 110
[1759] Leu Ser Leu Tyr Leu Asp Thr Gln Asp Tyr Cys His Leu Thr Leu Lys
[1760] 115 120 125
[1761] Phe Tyr Asp Asp Gly Ser Glu Ala Ile Ser Ala Leu Gln Glu Leu Ala
[1762] 130 135 140
[1763] Leu Ala Pro Asp Leu Ala Ala Gln Ile Gln Phe Glu Lys Gln Gln Phe

[1764] 145 150 155 160
[1765] Asp Glu Leu Val Val Lys Lys Ser Phe Lys Leu Ser Leu Leu Ser Arg
[1766] 165 170 175
[1767] Tyr Phe Trp Gly Lys Leu Phe Glu Ser Glu Tyr Ile Trp Phe Asn Gln
[1768] 180 185 190
[1769] Ala Ile Leu Gln Lys Ala Glu Leu Gln Ile Leu Lys Gln Glu Ile Ser
[1770] 195 200 205
[1771] Ser Ser Arg Gln Met Asp Phe Ala Ile Tyr Gln Gln Met Ser Asp Glu
[1772] 210 215 220
[1773] Gln Lys Gln Leu Val Leu Glu Ile Leu Asn Ile Asp Leu Asn Lys Val
[1774] 225 230 235 240
[1775] Ala Tyr Leu Lys Gln Leu Met Glu Asn Gln Pro Ser Phe Leu Phe Leu
[1776] 245 250 255
[1777] Gly Thr Thr Leu Phe Asn Ile Thr Gln Glu Thr Lys Thr Trp Leu Met
[1778] 260 265 270
[1779] Gln Met His Val Asp Leu Ile Gln Gln Tyr Cys Leu Pro Ser Gly Gln
[1780] 275 280 285
[1781] Phe Phe Asn Asn Lys Ala Gly Tyr Leu Cys Phe Tyr Lys Gly His Pro
[1782] 290 295 300
[1783] Asn Glu Lys Glu Met Asn Gln Met Ile Leu Ser Gln Phe Lys Asn Leu
[1784] 305 310 315 320
[1785] Ile Ala Leu Pro Asp Asp Ile Pro Leu Glu Ile Leu Leu Leu Leu Gly
[1786] 325 330 335
[1787] Val Ile Pro Ser Lys Val Gly Gly Phe Ala Ser Ser Ala Leu Phe Asn
[1788] 340 345 350
[1789] Phe Thr Pro Ala Gln Ile Glu Asn Ile Ile Phe Phe Thr Pro Arg Tyr
[1790] 355 360 365
[1791] Phe Glu Lys Asp Asn Arg Leu His Ala Thr Gln Tyr Arg Leu Met Gln
[1792] 370 375 380
[1793] Gly Leu Ile Glu Leu Gly Tyr Leu Asp Ala Glu Lys Ser Val Thr His
[1794] 385 390 395 400
[1795] Phe Glu Ile Met Gln Leu Leu Thr Lys Glu
[1796] 405 410
[1797] <210> 52
[1798] <211> 406
[1799] <212> PRT
[1800] <213> 副溶血嗜血杆菌(Haemophilus parahaemolyticus)
[1801] <400> 52
[1802] Met Thr Glu Gln Tyr Ile Lys Asn Val Glu Val Tyr Leu Asp Tyr Ala
[1803] 1 5 10 15
[1804] Thr Ile Pro Thr Leu Asn Tyr Phe Tyr His Phe Thr Glu Asn Lys Asp
[1805] 20 25 30

[1806] Asp Ile Ala Thr Ile Arg Leu Phe Gly Leu Gly Arg Phe Asn Ile Ser
[1807] 35 40 45
[1808] Lys Ser Ile Ile Glu Ser Tyr Pro Glu Gly Ile Ile Arg Tyr Cys Pro
[1809] 50 55 60
[1810] Ile Ile Phe Glu Asp Gln Thr Ala Phe Gln Gln Leu Phe Ile Thr Leu
[1811] 65 70 75 80
[1812] Leu Thr Glu Asp Ser Phe Cys Gln Tyr Arg Phe Asn Phe His Ile Asn
[1813] 85 90 95
[1814] Leu Phe His Ser Trp Lys Met Leu Ile Pro Leu Leu His Ile Ile Trp
[1815] 100 105 110
[1816] Gln Phe Lys His Lys Val Leu Asp Ile Lys Leu Asn Phe Tyr Asp Asp
[1817] 115 120 125
[1818] Gly Ser Glu Gly Leu Val Thr Leu Ser Lys Ile Glu Gln Asn Tyr Ser
[1819] 130 135 140
[1820] Ser Glu Ile Leu Gln Lys Ile Ile Asp Ile Asp Ser Gln Ser Phe Tyr
[1821] 145 150 155 160
[1822] Ala Asp Lys Leu Ser Phe Leu Asp Glu Asp Ile Ala Arg Tyr Leu Trp
[1823] 165 170 175
[1824] Asn Ser Leu Phe Glu Ser His Tyr Tyr Leu Leu Asn Asp Phe Leu Leu
[1825] 180 185 190
[1826] Lys Asn Glu Lys Leu Ser Leu Leu Lys Asn Ser Ile Lys Tyr Cys His
[1827] 195 200 205
[1828] Ile Met Asp Leu Glu Arg Tyr Leu Gln Phe Thr Gln Glu Glu Lys Asp
[1829] 210 215 220
[1830] Phe Phe Asn Glu Leu Leu Gly Ile Asn Ile Gln Ser Leu Glu Asp Lys
[1831] 225 230 235 240
[1832] Ile Lys Ile Phe Gln Gln Lys Lys Thr Phe Ile Phe Thr Gly Thr Thr
[1833] 245 250 255
[1834] Ile Phe Ser Leu Pro Lys Glu Glu Glu Glu Thr Leu Tyr Arg Leu His
[1835] 260 265 270
[1836] Leu Asn Ala Ile Leu Asn Tyr Ile His Pro Asn Gly Lys Tyr Phe Ile
[1837] 275 280 285
[1838] Gly Asp Gly Phe Thr Leu Val Ile Lys Gly His Pro His Gln Lys Glu
[1839] 290 295 300
[1840] Met Asn Ser Arg Leu Glu Lys Ser Phe Glu Lys Ala Val Met Leu Pro
[1841] 305 310 315 320
[1842] Asp Asn Ile Pro Phe Glu Ile Leu Tyr Leu Ile Gly Cys Lys Pro Asp
[1843] 325 330 335
[1844] Lys Ile Gly Gly Phe Val Ser Thr Ser Tyr Phe Ser Cys Asp Lys Lys
[1845] 340 345 350
[1846] Asn Ile Ala Asp Leu Leu Phe Ile Ser Ala Arg Gln Glu Glu Val Arg
[1847] 355 360 365

[1848] Lys Asn Asp Tyr Leu Phe Asn Ile Gln Tyr Gln Leu Arg Asp Met Met
[1849] 370 375 380
[1850] Ile Lys Thr Gly Phe Ile Gln Glu Glu Lys Thr His Phe Tyr Ser Asp
[1851] 385 390 395 400
[1852] Ile Pro Ile Phe Ile Ser
[1853] 405
[1854] <210> 53
[1855] <211> 300
[1856] <212> PRT
[1857] <213> 睡眠嗜血杆菌(Haemophilus somnus)
[1858] <400> 53
[1859] Met Lys Tyr Asn Ile Lys Ile Lys Ala Ile Val Ile Val Ser Ser Leu
[1860] 1 5 10 15
[1861] Arg Met Leu Leu Ile Phe Leu Met Leu Asn Lys Tyr His Leu Asp Glu
[1862] 20 25 30
[1863] Val Leu Phe Val Phe Asn Glu Gly Phe Glu Leu His Lys Lys Tyr Lys
[1864] 35 40 45
[1865] Ile Lys His Tyr Val Ala Ile Lys Lys Lys Ile Thr Lys Phe Trp Arg
[1866] 50 55 60
[1867] Leu Tyr Tyr Lys Leu Tyr Phe Tyr Arg Phe Lys Ile Asp Arg Ile Pro
[1868] 65 70 75 80
[1869] Val Tyr Gly Ala Asp His Leu Gly Trp Thr Asp Tyr Phe Leu Lys Tyr
[1870] 85 90 95
[1871] Phe Asp Phe Tyr Leu Ile Glu Asp Gly Ile Ala Asn Phe Ser Pro Lys
[1872] 100 105 110
[1873] Arg Tyr Glu Ile Asn Leu Thr Arg Asn Ile Pro Val Phe Gly Phe His
[1874] 115 120 125
[1875] Lys Thr Val Lys Lys Ile Tyr Leu Thr Ser Leu Glu Asn Val Pro Ser
[1876] 130 135 140
[1877] Asp Ile Arg His Lys Val Glu Leu Ile Ser Leu Glu His Leu Trp Lys
[1878] 145 150 155 160
[1879] Thr Arg Thr Ala Gln Glu Gln His Asn Ile Leu Asp Phe Phe Ala Phe
[1880] 165 170 175
[1881] Asn Leu Asp Ser Leu Ile Ser Leu Lys Met Lys Lys Tyr Ile Leu Phe
[1882] 180 185 190
[1883] Thr Gln Cys Leu Ser Glu Asp Arg Val Ile Ser Glu Gln Glu Lys Ile
[1884] 195 200 205
[1885] Ala Ile Tyr Gln His Ile Ile Lys Asn Tyr Asp Glu Arg Leu Leu Val
[1886] 210 215 220
[1887] Ile Lys Pro His Pro Arg Glu Thr Thr Asp Tyr Gln Lys Tyr Phe Glu
[1888] 225 230 235 240
[1889] Asn Val Phe Val Tyr Gln Asp Val Val Pro Ser Glu Leu Phe Glu Leu

[1890] 245 250 255
[1891] Leu Asp Val Asn Phe Glu Arg Val Ile Thr Leu Phe Ser Thr Ala Val
[1892] 260 265 270
[1893] Phe Lys Tyr Asp Arg Asn Ile Val Asp Phe Tyr Gly Thr Arg Ile His
[1894] 275 280 285
[1895] Asp Lys Ile Tyr Gln Trp Phe Gly Asp Ile Lys Phe
[1896] 290 295 300
[1897] <210> 54
[1898] <211> 381
[1899] <212> PRT
[1900] <213> 哈氏弧菌(Vibrio harveyi)
[1901] <400> 54
[1902] Met Asp Ser Ser Pro Glu Asn Thr Ser Ser Thr Leu Glu Ile Tyr Ile
[1903] 1 5 10 15
[1904] Asp Ser Ala Thr Leu Pro Ser Leu Gln His Met Val Lys Ile Ile Asp
[1905] 20 25 30
[1906] Glu Gln Ser Gly Asn Lys Lys Leu Ile Asn Trp Lys Arg Tyr Pro Ile
[1907] 35 40 45
[1908] Asp Asp Glu Leu Leu Leu Asp Lys Ile Asn Ala Leu Ser Phe Ser Asp
[1909] 50 55 60
[1910] Thr Thr Asp Leu Thr Arg Tyr Met Glu Ser Ile Leu Leu Ile Gly Asp
[1911] 65 70 75 80
[1912] Ile Lys Arg Val Val Ile Asn Gly Asn Ser Leu Ser Asn Tyr Asn Ile
[1913] 85 90 95
[1914] Val Gly Val Met Arg Ser Ile Asn Ala Leu Gly Leu Asp Leu Asp Val
[1915] 100 105 110
[1916] Glu Ile Asn Phe Tyr Asp Asp Gly Ser Ala Glu Tyr Val Arg Leu Tyr
[1917] 115 120 125
[1918] Asn Phe Ser Gln Leu Pro Glu Ala Glu Arg Glu Leu Leu Val Ser Met
[1919] 130 135 140
[1920] Ser Lys Asn Asn Ile Leu Ala Ala Val Asn Gly Ile Gly Ser Tyr Asp
[1921] 145 150 155 160
[1922] Ser Gly Ser Pro Glu Asn Ile Tyr Gly Phe Ala Gln Ile Tyr Pro Ala
[1923] 165 170 175
[1924] Thr Tyr His Met Leu Arg Ala Asp Ile Phe Asp Thr Asp Leu Glu Ile
[1925] 180 185 190
[1926] Gly Leu Ile Arg Asp Ile Leu Gly Asp Asn Val Lys Gln Met Lys Trp
[1927] 195 200 205
[1928] Gly Gln Phe Leu Gly Phe Asn Glu Glu Gln Lys Glu Leu Phe Tyr Gln
[1929] 210 215 220
[1930] Leu Thr Ser Phe Asn Pro Asp Lys Ile Gln Ala Gln Tyr Lys Glu Ser
[1931] 225 230 235 240

[1932] Pro Asn Lys Asn Phe Val Phe Val Gly Thr Asn Ser Arg Ser Ala Thr
[1933] 245 250 255
[1934] Ala Glu Gln Gln Ile Asn Ile Ile Lys Glu Ala Lys Lys Leu Asp Ser
[1935] 260 265 270
[1936] Glu Ile Ile Pro Asn Ser Ile Asp Gly Tyr Asp Leu Phe Phe Lys Gly
[1937] 275 280 285
[1938] His Pro Ser Ala Thr Tyr Asn Gln Gln Ile Val Asp Ala His Asp Met
[1939] 290 295 300
[1940] Thr Glu Ile Tyr Asn Arg Thr Pro Phe Glu Val Leu Ala Met Thr Ser
[1941] 305 310 315 320
[1942] Ser Leu Pro Asp Ala Val Gly Gly Met Gly Ser Ser Leu Phe Phe Ser
[1943] 325 330 335
[1944] Leu Pro Lys Thr Val Glu Thr Lys Phe Ile Phe Tyr Lys Ser Gly Thr
[1945] 340 345 350
[1946] Asp Ile Glu Ser Asn Ala Leu Ile Gln Val Met Leu Lys Leu Gly Ile
[1947] 355 360 365
[1948] Ile Thr Asp Glu Lys Val Arg Phe Thr Thr Asp Ile Lys
[1949] 370 375 380
[1950] <210> 55
[1951] <211> 483
[1952] <212> PRT
[1953] <213> 别样杆菌属种(Alistipes sp.)
[1954] <400> 55
[1955] Met Ala Ser Cys Ser Asp Asp Asp Lys Glu Gln Thr Gly Phe Gln Ile
[1956] 1 5 10 15
[1957] Asp Asp Gly Ser Gly Phe Leu Ser Leu Asp Ala Ala Ala Arg Ser Gly
[1958] 20 25 30
[1959] Ser Ile Ala Ile Thr Ala Asn Asn Ser Trp Ser Val Thr Gln Asp Lys
[1960] 35 40 45
[1961] Asp Ser Glu Trp Leu Thr Leu Ser Thr Thr Ser Gly Ala Ala Gly Arg
[1962] 50 55 60
[1963] Thr Glu Ile Gly Ile Met Leu Glu Ala Asn Pro Gly Glu Ala Arg Asn
[1964] 65 70 75 80
[1965] Ala Gly Leu Thr Phe Asn Ser Gly Gly Arg Thr Tyr Pro Phe Val Ile
[1966] 85 90 95
[1967] Thr Gln Ser Ala His Val Thr Ala Asp Phe Asp Asp Ala Asp His Cys
[1968] 100 105 110
[1969] Phe Tyr Ile Thr Phe Gly Thr Leu Pro Thr Leu Tyr Ala Gly Leu His
[1970] 115 120 125
[1971] Val Leu Ser His Asp Lys Pro Ser Tyr Val Phe Phe Gln Arg Ser Gln
[1972] 130 135 140
[1973] Thr Phe Arg Pro Glu Glu Phe Pro Ala His Ala Glu Val Thr Ile Ala

[1974] 145 150 155 160
[1975] Ala Asp Pro Ser Ala Asn Ala Thr Asp Glu Asp Met Glu Arg Met Arg
[1976] 165 170 175
[1977] Thr Ala Met Lys Gln Gln Ile Leu Lys Ile Asn Val Glu Asp Pro Thr
[1978] 180 185 190
[1979] Ala Val Phe Gly Leu Tyr Val Asp Asp Leu Arg Cys Gly Ile Gly Tyr
[1980] 195 200 205
[1981] Asp Trp Phe Val Ala Gln Gly Ile Asp Ser Thr Arg Val Lys Val Ser
[1982] 210 215 220
[1983] Met Leu Ser Asp Gly Thr Gly Thr Tyr Asn Asn Phe Tyr Asn Tyr Phe
[1984] 225 230 235 240
[1985] Gly Asp Pro Ala Thr Ala Glu Gln Asn Trp Glu Asn Tyr Ala Ala Gln
[1986] 245 250 255
[1987] Val Glu Ala Leu Asp Trp Gln His Gly Gly Arg Phe Pro Glu Thr Arg
[1988] 260 265 270
[1989] Met Pro Asp Gly Phe Asp Phe Tyr Glu Trp Pro Tyr Tyr Leu Ala Thr
[1990] 275 280 285
[1991] Arg Pro Asn Tyr Arg Leu Val Leu Gln Asp Asp Asp Leu Leu Glu Ala
[1992] 290 295 300
[1993] Thr Ser Pro Phe Met Thr Glu Arg Leu Gln Gln Met Arg Thr Glu Ser
[1994] 305 310 315 320
[1995] Lys Gln Pro Tyr Glu Leu Leu Ala Ser Leu Pro Ala Glu Ala Arg Gln
[1996] 325 330 335
[1997] Arg Phe Phe Arg Met Ala Gly Phe Asp Tyr Asp Ala Phe Ala Ala Leu
[1998] 340 345 350
[1999] Phe Asp Ala Ser Pro Lys Lys Asn Leu Val Ile Ile Gly Thr Ser His
[2000] 355 360 365
[2001] Thr Ser Glu Glu Ser Glu Ala Gln Gln Ala Ala Tyr Val Glu Arg Ile
[2002] 370 375 380
[2003] Ile Gly Asp Tyr Gly Thr Ala Tyr Asp Ile Phe Phe Lys Pro His Pro
[2004] 385 390 395 400
[2005] Ala Asp Ser Ser Ser Ser Asn Tyr Glu Glu Arg Phe Glu Gly Leu Thr
[2006] 405 410 415
[2007] Leu Leu Pro Gly Gln Met Pro Phe Glu Ile Phe Val Trp Ser Leu Leu
[2008] 420 425 430
[2009] Asp Lys Val Asp Leu Ile Gly Gly Tyr Ser Ser Thr Val Phe Leu Thr
[2010] 435 440 445
[2011] Val Pro Val Glu Lys Thr Gly Phe Ile Phe Ala Ala Asn Ala Glu Ser
[2012] 450 455 460
[2013] Leu Pro Arg Pro Leu Asn Val Leu Phe Arg Asn Ala Glu His Val Arg
[2014] 465 470 475 480
[2015] Trp Ile Gln

[2016] <210> 56
[2017] <211> 483
[2018] <212> PRT
[2019] <213> 沙氏别样杆菌(Alistipes shahii)
[2020] <400> 56
[2021] Met Asp Asp Gly Thr Pro Ser Val Ser Ile Asn Gly Gly Thr Asp Phe
[2022] 1 5 10 15
[2023] Leu Ser Leu Asp His Leu Ala Arg Ser Gly Lys Ile Thr Val Asn Ala
[2024] 20 25 30
[2025] Pro Ala Pro Trp Ser Val Thr Leu Ala Pro Glu Asn Tyr Gly Gln Asp
[2026] 35 40 45
[2027] Glu Lys Pro Asp Trp Leu Thr Leu Ser Ala Glu Glu Gly Pro Ala Gly
[2028] 50 55 60
[2029] Tyr Ser Glu Ile Asp Val Thr Phe Ala Glu Asn Pro Gly Pro Ala Arg
[2030] 65 70 75 80
[2031] Ser Ala Ser Leu Leu Phe Ser Cys Asp Gly Lys Thr Leu Ala Phe Thr
[2032] 85 90 95
[2033] Val Ser Gln Ser Ala Gly Gly Thr Gly Phe Asp Ala Pro Asp Tyr Tyr
[2034] 100 105 110
[2035] Phe Tyr Ile Ser Val Gly Thr Met Pro Thr Leu Tyr Ser Gly Leu His
[2036] 115 120 125
[2037] Leu Leu Ser His Asp Lys Pro Ser Tyr Val Ser Tyr Glu Arg Ala Ser
[2038] 130 135 140
[2039] Thr Phe Asp Ala Ala Glu Phe Pro Asp Arg Ala Phe Val Tyr Pro Val
[2040] 145 150 155 160
[2041] Ala Asp Pro Thr Gly His Ala Thr Asn Glu Glu Leu Arg Ala Met Ser
[2042] 165 170 175
[2043] Glu Ala Met Lys Arg Arg Ile Leu Glu Ile Asn Ala Glu Asp Pro Thr
[2044] 180 185 190
[2045] Ala Val Phe Gly Leu Trp Val Asp Asp Leu Arg Cys Arg Leu Gly Tyr
[2046] 195 200 205
[2047] Asp Trp Phe Val Ala Gln Gly Ile Asp Ser Ala Arg Val Lys Val Thr
[2048] 210 215 220
[2049] Met Leu Ser Asp Gly Thr Ala Thr Tyr Asn Asn Phe His Asn Tyr Phe
[2050] 225 230 235 240
[2051] Gly Asp Ala Ala Thr Ala Glu Gln Asn Trp Asn Asp Tyr Ala Ala Glu
[2052] 245 250 255
[2053] Val Glu Ala Leu Asp Trp Asn His Gly Gly Arg Tyr Pro Glu Thr Arg
[2054] 260 265 270
[2055] Ala Pro Glu Glu Phe Ala Ser Tyr Thr Trp Pro Tyr Tyr Leu Ser Thr
[2056] 275 280 285
[2057] Arg Pro Asp Tyr Arg Leu Met Leu Gln Asn Ser Ser Leu Met Glu Ser

[2058] 290 295 300
[2059] Ser Cys Pro Phe Ile Ala Asp Arg Leu Ala Ala Met Lys Met Glu Ser
[2060] 305 310 315 320
[2061] Val Gln Pro Tyr Glu Leu Leu Thr Ala Leu Pro Glu Ala Ser Lys Gln
[2062] 325 330 335
[2063] Gln Phe Tyr Arg Met Ala Lys Phe Asp Tyr Ala Arg Phe Ala Gly Leu
[2064] 340 345 350
[2065] Phe Asp Leu Ser Pro Lys Lys Asn Leu Ile Ile Ile Gly Thr Ser His
[2066] 355 360 365
[2067] Ser Ser Ala Ala Ser Glu Gln Gln Gln Ala Ala Tyr Val Glu Arg Ile
[2068] 370 375 380
[2069] Ile Gln Gln Tyr Gly Ser Asp Tyr Asp Ile Phe Phe Lys Pro His Pro
[2070] 385 390 395 400
[2071] Ala Asp Ser Ser Ser Ala Gly Tyr Pro Asp Arg Phe Glu Gly Leu Thr
[2072] 405 410 415
[2073] Leu Leu Pro Gly Gln Met Pro Phe Glu Ile Phe Val Trp Ala Leu Leu
[2074] 420 425 430
[2075] Asp Lys Ile Asp Met Ile Gly Gly Tyr Pro Ser Thr Thr Phe Ile Ser
[2076] 435 440 445
[2077] Val Pro Leu Asp Lys Val Gly Phe Leu Phe Ala Ala Asp Ala Asp Gly
[2078] 450 455 460
[2079] Leu Val Arg Pro Leu Asn Ile Leu Phe Arg Asp Ala Ala Asn Val Glu
[2080] 465 470 475 480
[2081] Trp Ile Gln
[2082] <210> 57
[2083] <211> 401
[2084] <212> PRT
[2085] <213> 猪放线杆菌(Actinobacillus suis)
[2086] <400> 57
[2087] Met Glu Arg Thr Pro Gln Leu Gln Ala Val Asp Ile Tyr Ile Asp Phe
[2088] 1 5 10 15
[2089] Ala Thr Ile Pro Ser Leu Ser Tyr Phe Leu His Phe Leu Lys His Lys
[2090] 20 25 30
[2091] His Asp Asp Gln Arg Leu Arg Leu Phe Ser Leu Ala Arg Phe Glu Met
[2092] 35 40 45
[2093] Pro Gln Thr Leu Ile Glu Gln Tyr Glu Gly Ile Ile Gln Phe Ser Arg
[2094] 50 55 60
[2095] Asn Val Glu His Asn Val Glu Pro Leu Leu Glu Gln Leu Gln Thr Ile
[2096] 65 70 75 80
[2097] Leu Ser Gln Glu Gly Lys Gln Phe Glu Leu His Leu His Leu Asn Leu
[2098] 85 90 95
[2099] Phe His Ser Phe Glu Met Phe Leu Asn Leu Ser Pro Thr Tyr Thr Gln

[2100] 100 105 110
[2101] Tyr Lys Glu Lys Ile Ser Lys Ile Val Leu His Leu Tyr Asp Asp Gly
[2102] 115 120 125
[2103] Ser Glu Gly Val Met Lys Gln Tyr Gln Leu Gln Lys Ser Ser Ser Leu
[2104] 130 135 140
[2105] Val Gln Asp Leu Ala Ala Thr Lys Ala Ser Leu Val Ser Leu Phe Glu
[2106] 145 150 155 160
[2107] Asn Gly Glu Gly Ser Phe Ser Gln Ile Asp Leu Ile Arg Tyr Val Trp
[2108] 165 170 175
[2109] Asn Ala Val Leu Glu Thr His Tyr Tyr Leu Leu Ser Asp His Phe Leu
[2110] 180 185 190
[2111] Leu Asp Glu Lys Leu Gln Pro Leu Lys Ala Glu Leu Gly His Tyr Gln
[2112] 195 200 205
[2113] Leu Leu Asn Leu Ser Ala Tyr Gln Tyr Leu Ser Ser Glu Asp Leu Leu
[2114] 210 215 220
[2115] Trp Leu Lys Gln Ile Leu Lys Ile Asp Thr Glu Leu Glu Ser Leu Met
[2116] 225 230 235 240
[2117] Gln Lys Leu Thr Ala Gln Pro Val Tyr Phe Phe Ser Gly Thr Thr Phe
[2118] 245 250 255
[2119] Phe Asn Ile Ser Phe Glu Asp Lys Gln Arg Leu Ala Asn Ile His Ala
[2120] 260 265 270
[2121] Ile Leu Ile Arg Glu His Leu Asp Pro Asn Ser Gln Leu Phe Ile Gly
[2122] 275 280 285
[2123] Glu Pro Tyr Leu Phe Val Phe Lys Gly His Pro Asn Ser Pro Glu Ile
[2124] 290 295 300
[2125] Asn Gln Ala Leu Arg Glu Tyr Tyr Pro Asn Val Ile Phe Leu Pro Glu
[2126] 305 310 315 320
[2127] Asn Ile Pro Phe Glu Ile Leu Thr Leu Leu Gly Phe Ser Pro Gln Lys
[2128] 325 330 335
[2129] Ile Gly Gly Phe Ala Ser Thr Ile His Val Asn Ser Glu Gln Ser Lys
[2130] 340 345 350
[2131] Leu Ala Lys Leu Phe Phe Leu Thr Ser Thr Asp Glu Gln Glu Arg Gln
[2132] 355 360 365
[2133] Leu Ser Asp Gly Tyr Ile Lys Gln Tyr Ala Leu Ala Gln Ala Met Leu
[2134] 370 375 380
[2135] Glu Met Gln Leu Val Ser Gln Glu Gln Val Tyr Tyr Cys Ser Leu Ser
[2136] 385 390 395 400
[2137] Ser
[2138] <210> 58
[2139] <211> 401
[2140] <212> PRT
[2141] <213> 荚膜放线杆菌(Actinobacillus capsulatus)

[2142] <400> 58

[2143] Met Glu Arg Ile Pro Gln Leu Gln Ala Val Asp Ile Tyr Ile Asp Phe
[2144] 1 5 10 15
[2145] Ala Thr Ile Pro Ser Leu Ser Tyr Phe Leu His Phe Leu Lys His Lys
[2146] 20 25 30
[2147] His Asp His Gln Arg Leu Arg Leu Phe Ser Leu Ala Arg Phe Glu Met
[2148] 35 40 45
[2149] Pro Gln Thr Val Ile Glu Gln Tyr Glu Gly Ile Ile Gln Phe Ser Arg
[2150] 50 55 60
[2151] Asn Val Glu His Asn Val Glu Gln Leu Leu Glu Gln Leu Gln Thr Ile
[2152] 65 70 75 80
[2153] Leu Ser Gln Glu Gly Lys Gln Phe Glu Leu His Leu His Leu Asn Leu
[2154] 85 90 95
[2155] Phe His Ser Phe Glu Met Phe Leu Asn Leu Ser Pro Thr Tyr Thr Lys
[2156] 100 105 110
[2157] Tyr Lys Glu Lys Ile Ser Lys Ile Val Leu His Leu Tyr Asp Asp Gly
[2158] 115 120 125
[2159] Ser Glu Gly Val Met Lys Gln Tyr Gln Leu Gln Gln Ser Asn Ser Leu
[2160] 130 135 140
[2161] Ala Gln Asp Leu Ala Ser Thr Lys Ala Ser Leu Val Ser Leu Phe Lys
[2162] 145 150 155 160
[2163] Asn Gly Glu Gly Ala Phe Ser Gln Ile Asp Leu Ile Arg Tyr Val Trp
[2164] 165 170 175
[2165] Asn Ala Val Leu Glu Thr His Tyr Tyr Leu Leu Ser Asp His Phe Leu
[2166] 180 185 190
[2167] Ala His Glu Lys Leu Gln Pro Leu Lys Ile Glu Leu Gly His Tyr Gln
[2168] 195 200 205
[2169] Leu Leu Asn Leu Ser Ala Tyr Gln Tyr Leu Ser Ser Glu Asp Leu Leu
[2170] 210 215 220
[2171] Trp Leu Lys Gln Ile Leu Lys Ile Asp Ala Glu Leu Glu Ser Leu Met
[2172] 225 230 235 240
[2173] His Lys Leu Thr Thr Gln Pro Val Tyr Phe Phe Ser Gly Thr Thr Phe
[2174] 245 250 255
[2175] Phe Asn Ile Ser Phe Glu Asp Lys Gln Arg Leu Ala Asn Ile His Ala
[2176] 260 265 270
[2177] Ile Leu Ile Arg Glu His Leu Asp Pro Asn Ser Gln Leu Phe Ile Gly
[2178] 275 280 285
[2179] Glu Pro Tyr Leu Phe Val Phe Lys Gly His Pro Asn Ser Pro Glu Ile
[2180] 290 295 300
[2181] Asn Gln Ala Leu Arg Glu Tyr Tyr Pro Asn Ala Ile Phe Leu Pro Glu
[2182] 305 310 315 320
[2183] Asn Ile Pro Phe Glu Ile Leu Thr Leu Leu Gly Phe Ser Pro Gln Lys

[2184] 325 330 335
[2185] Ile Gly Gly Phe Ala Ser Thr Ile His Val Asn Ser Glu Gln Ser Lys
[2186] 340 345 350
[2187] Leu Ala Lys Leu Phe Phe Leu Thr Ser Thr Asp Glu Gln Glu Arg Asn
[2188] 355 360 365
[2189] Arg Ser Asp Gly Tyr Ile Lys Gln Tyr Ala Leu Ala Gln Ala Met Leu
[2190] 370 375 380
[2191] Glu Met Gln Leu Val Ser Gln Glu Gln Val Tyr Tyr Cys Ser Leu Ser
[2192] 385 390 395 400
[2193] Ser
[2194] <210> 59
[2195] <211> 311
[2196] <212> PRT
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[2198] <400> 59
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[2201] Gln Val Ile Ile Ala Glu Lys Ile Ile Glu Arg Tyr Pro Glu Gln Lys
[2202] 20 25 30
[2203] Phe Tyr Gly Val Met Leu Glu Ser Phe Tyr Asn Asp Lys Phe Asp Phe
[2204] 35 40 45
[2205] Tyr Glu Asn Lys Leu Lys His Leu Cys His Glu Phe Phe Cys Ile Lys
[2206] 50 55 60
[2207] Ile Ala Arg Phe Lys Leu Glu Arg Tyr Lys Asn Leu Leu Ser Leu Leu
[2208] 65 70 75 80
[2209] Lys Ile Lys Asn Lys Thr Phe Asp Arg Val Phe Leu Ala Asn Ile Glu
[2210] 85 90 95
[2211] Lys Arg Tyr Ile His Ile Ile Leu Ser Asn Ile Phe Phe Lys Glu Leu
[2212] 100 105 110
[2213] Tyr Thr Phe Asp Asp Gly Thr Ala Asn Ile Ala Pro Asn Ser His Leu
[2214] 115 120 125
[2215] Tyr Gln Glu Tyr Asp His Ser Leu Lys Lys Arg Ile Thr Asp Ile Leu
[2216] 130 135 140
[2217] Leu Pro Asn His Tyr Asn Ser Asn Lys Val Lys Asn Ile Ser Lys Leu
[2218] 145 150 155 160
[2219] His Tyr Ser Ile Tyr Arg Cys Lys Asn Asn Ile Ile Asp Asn Ile Glu
[2220] 165 170 175
[2221] Tyr Met Pro Leu Phe Asn Leu Glu Lys Lys Tyr Thr Ala Gln Asp Lys
[2222] 180 185 190
[2223] Ser Ile Ser Ile Leu Leu Gly Gln Pro Ile Phe Tyr Asp Glu Glu Lys
[2224] 195 200 205
[2225] Asn Ile Arg Leu Ile Lys Glu Val Ile Ala Lys Phe Lys Ile Asp Tyr

[2226] 210 215 220
[2227] Tyr Phe Pro His Pro Arg Glu Asp Tyr Tyr Ile Asp Asn Val Ser Tyr
[2228] 225 230 235 240
[2229] Ile Lys Thr Pro Leu Ile Phe Glu Glu Phe Tyr Ala Glu Arg Ser Ile
[2230] 245 250 255
[2231] Glu Asn Ser Ile Lys Ile Tyr Thr Phe Phe Ser Ser Ala Val Leu Asn
[2232] 260 265 270
[2233] Ile Val Thr Lys Glu Asn Ile Asp Arg Ile Tyr Ala Leu Lys Pro Lys
[2234] 275 280 285
[2235] Leu Thr Glu Lys Ala Tyr Leu Asp Cys Tyr Asp Ile Leu Lys Asp Phe
[2236] 290 295 300
[2237] Gly Ile Lys Val Ile Asp Ile
[2238] 305 310
[2239] <210> 60
[2240] <211> 399
[2241] <212> PRT
[2242] <213> 杜克雷嗜血杆菌(Haemophilus ducreyi)
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[2245] 1 5 10 15
[2246] Pro Ser Leu Ala Cys Phe Met His Phe Ile Gln His Lys Asp Asp Val
[2247] 20 25 30
[2248] Asp Ser Ile Arg Leu Phe Gly Leu Ala Arg Phe Asp Ile Pro Gln Ser
[2249] 35 40 45
[2250] Ile Ile Asp Arg Tyr Pro Ala Asn His Leu Phe Tyr His Asn Ile Asp
[2251] 50 55 60
[2252] Asn Arg Asp Leu Thr Ala Val Leu Asn Gln Leu Ala Asp Ile Leu Ala
[2253] 65 70 75 80
[2254] Gln Glu Asn Lys Arg Phe Gln Ile Asn Leu His Leu Asn Leu Phe His
[2255] 85 90 95
[2256] Ser Ile Asp Leu Phe Phe Ala Ile Tyr Pro Ile Tyr Gln Gln Tyr Gln
[2257] 100 105 110
[2258] His Lys Ile Ser Thr Ile Gln Leu Gln Leu Tyr Asp Asp Gly Ser Glu
[2259] 115 120 125
[2260] Gly Ile Val Thr Gln His Ser Leu Cys Lys Ile Ala Asp Leu Glu Gln
[2261] 130 135 140
[2262] Leu Ile Leu Gln His Lys Asn Val Leu Leu Glu Leu Leu Thr Lys Gly
[2263] 145 150 155 160
[2264] Thr Ala Asn Val Pro Asn Pro Thr Leu Leu Arg Tyr Leu Trp Asn Asn
[2265] 165 170 175
[2266] Ile Ile Asp Ser Gln Phe His Leu Ile Ser Asp His Phe Leu Gln His
[2267] 180 185 190

[2268] Pro Lys Leu Gln Pro Leu Lys Arg Leu Leu Lys Arg Tyr Thr Ile Leu
[2269] 195 200 205
[2270] Asp Phe Thr Cys Tyr Pro Arg Phe Asn Ala Glu Gln Lys Gln Leu Leu
[2271] 210 215 220
[2272] Lys Glu Ile Leu His Ile Ser Asn Glu Leu Glu Asn Leu Leu Lys Leu
[2273] 225 230 235 240
[2274] Leu Lys Gln His Asn Thr Phe Leu Phe Thr Gly Thr Thr Ala Phe Asn
[2275] 245 250 255
[2276] Leu Asp Gln Glu Lys Leu Asp Leu Leu Thr Gln Leu His Ile Leu Leu
[2277] 260 265 270
[2278] Leu Asn Glu His Gln Asn Pro His Ser Thr His Tyr Ile Gly Asn Asn
[2279] 275 280 285
[2280] Tyr Leu Leu Leu Ile Lys Gly His Ala Asn Ser Pro Ala Leu Asn His
[2281] 290 295 300
[2282] Thr Leu Ala Leu His Phe Pro Asp Ala Ile Phe Leu Pro Ala Asn Ile
[2283] 305 310 315 320
[2284] Pro Phe Glu Ile Phe Ala Met Leu Gly Phe Thr Pro Asn Lys Met Gly
[2285] 325 330 335
[2286] Gly Phe Ala Ser Thr Ser Tyr Ile Asn Tyr Pro Thr Glu Asn Ile Asn
[2287] 340 345 350
[2288] His Leu Phe Phe Leu Thr Ser Asp Gln Pro Ser Ile Arg Thr Lys Trp
[2289] 355 360 365
[2290] Leu Asp Tyr Glu Lys Gln Phe Gly Leu Met Tyr Ser Leu Leu Ala Met
[2291] 370 375 380
[2292] Gln Lys Ile Asn Glu Asp Gln Ala Phe Met Cys Thr Ile His Asn
[2293] 385 390 395
[2294] <210> 61
[2295] <211> 497
[2296] <212> PRT
[2297] <213> 鳃鱼发光杆菌(Photobacterium leiognathi)
[2298] <400> 61
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[2301] Asn Asp Asn Val Ile Glu Asn Asn Thr Tyr Gln Val Lys Pro Ile Asp
[2302] 20 25 30
[2303] Thr Pro Thr Thr Phe Asp Ser Tyr Ser Trp Ile Gln Thr Cys Gly Thr
[2304] 35 40 45
[2305] Pro Ile Leu Lys Asp Asp Glu Lys Tyr Ser Leu Ser Phe Asp Phe Val
[2306] 50 55 60
[2307] Ala Pro Glu Leu Asp Gln Asp Glu Lys Phe Cys Phe Glu Phe Thr Gly
[2308] 65 70 75 80
[2309] Asp Val Asp Gly Lys Arg Tyr Val Thr Gln Thr Asn Leu Thr Val Val

[2310] 85 90 95

[2311] Ala Pro Thr Leu Glu Val Tyr Val Asp His Ala Ser Leu Pro Ser Leu

[2312] 100 105 110

[2313] Gln Gln Leu Met Lys Ile Ile Gln Gln Lys Asn Glu Tyr Ser Gln Asn

[2314] 115 120 125

[2315] Glu Arg Phe Ile Ser Trp Gly Arg Ile Gly Leu Thr Glu Asp Asn Ala

[2316] 130 135 140

[2317] Glu Lys Leu Asn Ala His Ile Tyr Pro Leu Ala Gly Asn Asn Thr Ser

[2318] 145 150 155 160

[2319] Gln Glu Leu Val Asp Ala Val Ile Asp Tyr Ala Asp Ser Lys Asn Arg

[2320] 165 170 175

[2321] Leu Asn Leu Glu Leu Asn Thr Asn Thr Ala His Ser Phe Pro Asn Leu

[2322] 180 185 190

[2323] Ala Pro Ile Leu Arg Ile Ile Ser Ser Lys Ser Asn Ile Leu Ile Ser

[2324] 195 200 205

[2325] Asn Ile Asn Leu Tyr Asp Asp Gly Ser Ala Glu Tyr Val Asn Leu Tyr

[2326] 210 215 220

[2327] Asn Trp Lys Asp Thr Glu Asp Lys Ser Val Lys Leu Ser Asp Ser Phe

[2328] 225 230 235 240

[2329] Leu Val Leu Lys Asp Tyr Phe Asn Gly Ile Ser Ser Glu Lys Pro Ser

[2330] 245 250 255

[2331] Gly Ile Tyr Gly Arg Tyr Asn Trp His Gln Leu Tyr Asn Thr Ser Tyr

[2332] 260 265 270

[2333] Tyr Phe Leu Arg Lys Asp Tyr Leu Thr Val Glu Pro Gln Leu His Asp

[2334] 275 280 285

[2335] Leu Arg Glu Tyr Leu Gly Gly Ser Leu Lys Gln Met Ser Trp Asp Gly

[2336] 290 295 300

[2337] Phe Ser Gln Leu Ser Lys Gly Asp Lys Glu Leu Phe Leu Asn Ile Val

[2338] 305 310 315 320

[2339] Gly Phe Asp Gln Glu Lys Leu Gln Gln Glu Tyr Gln Gln Ser Glu Leu

[2340] 325 330 335

[2341] Pro Asn Phe Val Phe Thr Gly Thr Thr Thr Trp Ala Gly Gly Glu Thr

[2342] 340 345 350

[2343] Lys Glu Tyr Tyr Ala Gln Gln Gln Val Asn Val Val Asn Asn Ala Ile

[2344] 355 360 365

[2345] Asn Glu Thr Ser Pro Tyr Tyr Leu Gly Arg Glu His Asp Leu Phe Phe

[2346] 370 375 380

[2347] Lys Gly His Pro Arg Gly Gly Ile Ile Asn Asp Ile Ile Leu Gly Ser

[2348] 385 390 395 400

[2349] Phe Asn Asn Met Ile Asp Ile Pro Ala Lys Val Ser Phe Glu Val Leu

[2350] 405 410 415

[2351] Met Met Thr Gly Met Leu Pro Asp Thr Val Gly Gly Ile Ala Ser Ser

[2352] 420 425 430
[2353] Leu Tyr Phe Ser Ile Pro Ala Glu Lys Val Ser Phe Ile Val Phe Thr
[2354] 435 440 445
[2355] Ser Ser Asp Thr Ile Thr Asp Arg Glu Asp Ala Leu Lys Ser Pro Leu
[2356] 450 455 460
[2357] Val Gln Val Met Met Thr Leu Gly Ile Val Lys Glu Lys Asp Val Leu
[2358] 465 470 475 480
[2359] Phe Trp Ser Asp Leu Pro Asp Cys Ser Ser Gly Val Cys Ile Ala Gln
[2360] 485 490 495
[2361] Tyr
[2362] <210> 62
[2363] <211> 498
[2364] <212> PRT
[2365] <213> 发光杆菌属种(Photobacterium sp.)
[2366] <400> 62
[2367] Met Ser Glu Glu Asn Thr Gln Ser Ile Ile Lys Asn Asp Ile Asn Lys
[2368] 1 5 10 15
[2369] Thr Ile Ile Asp Glu Glu Tyr Val Asn Leu Glu Pro Ile Asn Gln Ser
[2370] 20 25 30
[2371] Asn Ile Ser Phe Thr Lys His Ser Trp Val Gln Thr Cys Gly Thr Gln
[2372] 35 40 45
[2373] Gln Leu Leu Thr Glu Gln Asn Lys Glu Ser Ile Ser Leu Ser Val Val
[2374] 50 55 60
[2375] Ala Pro Arg Leu Asp Asp Asp Glu Lys Tyr Cys Phe Asp Phe Asn Gly
[2376] 65 70 75 80
[2377] Val Ser Asn Lys Gly Glu Lys Tyr Ile Thr Lys Val Thr Leu Asn Val
[2378] 85 90 95
[2379] Val Ala Pro Ser Leu Glu Val Tyr Val Asp His Ala Ser Leu Pro Thr
[2380] 100 105 110
[2381] Leu Gln Gln Leu Met Asp Ile Ile Lys Ser Glu Glu Glu Asn Pro Thr
[2382] 115 120 125
[2383] Ala Gln Arg Tyr Ile Ala Trp Gly Arg Ile Val Pro Thr Asp Glu Gln
[2384] 130 135 140
[2385] Met Lys Glu Leu Asn Ile Thr Ser Phe Ala Leu Ile Asn Asn His Thr
[2386] 145 150 155 160
[2387] Pro Ala Asp Leu Val Gln Glu Ile Val Lys Gln Ala Gln Thr Lys His
[2388] 165 170 175
[2389] Arg Leu Asn Val Lys Leu Ser Ser Asn Thr Ala His Ser Phe Asp Asn
[2390] 180 185 190
[2391] Leu Val Pro Ile Leu Lys Glu Leu Asn Ser Phe Asn Asn Val Thr Val
[2392] 195 200 205
[2393] Thr Asn Ile Asp Leu Tyr Asp Asp Gly Ser Ala Glu Tyr Val Asn Leu

[2394] 210 215 220
[2395] Tyr Asn Trp Arg Asp Thr Leu Asn Lys Thr Asp Asn Leu Lys Ile Gly
[2396] 225 230 235 240
[2397] Lys Asp Tyr Leu Glu Asp Val Ile Asn Gly Ile Asn Glu Asp Thr Ser
[2398] 245 250 255
[2399] Asn Thr Gly Thr Ser Ser Val Tyr Asn Trp Gln Lys Leu Tyr Pro Ala
[2400] 260 265 270
[2401] Asn Tyr His Phe Leu Arg Lys Asp Tyr Leu Thr Leu Glu Pro Ser Leu
[2402] 275 280 285
[2403] His Glu Leu Arg Asp Tyr Ile Gly Asp Ser Leu Lys Gln Met Gln Trp
[2404] 290 295 300
[2405] Asp Gly Phe Lys Lys Phe Asn Ser Lys Gln Gln Glu Leu Phe Leu Ser
[2406] 305 310 315 320
[2407] Ile Val Asn Phe Asp Lys Gln Lys Leu Gln Asn Glu Tyr Asn Ser Ser
[2408] 325 330 335
[2409] Asn Leu Pro Asn Phe Val Phe Thr Gly Thr Thr Val Trp Ala Gly Asn
[2410] 340 345 350
[2411] His Glu Arg Glu Tyr Tyr Ala Lys Gln Gln Ile Asn Val Ile Asn Asn
[2412] 355 360 365
[2413] Ala Ile Asn Glu Ser Ser Pro His Tyr Leu Gly Asn Ser Tyr Asp Leu
[2414] 370 375 380
[2415] Phe Phe Lys Gly His Pro Gly Gly Gly Ile Ile Asn Thr Leu Ile Met
[2416] 385 390 395 400
[2417] Gln Asn Tyr Pro Ser Met Val Asp Ile Pro Ser Lys Ile Ser Phe Glu
[2418] 405 410 415
[2419] Val Leu Met Met Thr Asp Met Leu Pro Asp Ala Val Ala Gly Ile Ala
[2420] 420 425 430
[2421] Ser Ser Leu Tyr Phe Thr Ile Pro Ala Glu Lys Ile Lys Phe Ile Val
[2422] 435 440 445
[2423] Phe Thr Ser Thr Glu Thr Ile Thr Asp Arg Glu Thr Ala Leu Arg Ser
[2424] 450 455 460
[2425] Pro Leu Val Gln Val Met Ile Lys Leu Gly Ile Val Lys Glu Glu Asn
[2426] 465 470 475 480
[2427] Val Leu Phe Trp Ala Asp Leu Pro Asn Cys Glu Thr Gly Val Cys Ile
[2428] 485 490 495
[2429] Ala Val
[2430] <210> 63
[2431] <211> 482
[2432] <212> PRT
[2433] <213> 鲘鱼发光杆菌(Photobacterium leiognathi)
[2434] <400> 63
[2435] Met Asn Asp Asn Gln Asn Thr Val Asp Val Val Val Ser Thr Val Asn

[2436] 1 5 10 15
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[2438] 20 25 30
[2439] Pro Thr Thr Phe Asp Ser Tyr Ser Trp Ile Gln Thr Cys Gly Thr Pro
[2440] 35 40 45
[2441] Ile Leu Lys Asp Asp Glu Lys Tyr Ser Leu Ser Phe Asp Phe Val Ala
[2442] 50 55 60
[2443] Pro Glu Leu Asp Gln Asp Glu Lys Phe Cys Phe Glu Phe Thr Gly Asp
[2444] 65 70 75 80
[2445] Val Asp Gly Lys Arg Tyr Val Thr Gln Thr Asn Leu Thr Val Val Ala
[2446] 85 90 95
[2447] Pro Thr Leu Glu Val Tyr Val Asp His Ala Ser Leu Pro Ser Leu Gln
[2448] 100 105 110
[2449] Gln Leu Met Lys Ile Ile Gln Gln Lys Asn Glu Tyr Ser Gln Asn Glu
[2450] 115 120 125
[2451] Arg Phe Ile Ser Trp Gly Arg Ile Arg Leu Thr Glu Asp Asn Ala Glu
[2452] 130 135 140
[2453] Lys Leu Asn Ala His Ile Tyr Pro Leu Ala Gly Asn Asn Thr Ser Gln
[2454] 145 150 155 160
[2455] Glu Leu Val Asp Ala Val Ile Asp Tyr Ala Asp Ser Lys Asn Arg Leu
[2456] 165 170 175
[2457] Asn Leu Glu Leu Asn Thr Asn Thr Gly His Ser Phe Arg Asn Ile Ala
[2458] 180 185 190
[2459] Pro Ile Leu Arg Ala Thr Ser Ser Lys Asn Asn Ile Leu Ile Ser Asn
[2460] 195 200 205
[2461] Ile Asn Leu Tyr Asp Asp Gly Ser Ala Glu Tyr Val Ser Leu Tyr Asn
[2462] 210 215 220
[2463] Trp Lys Asp Thr Asp Asn Lys Ser Gln Lys Leu Ser Asp Ser Phe Leu
[2464] 225 230 235 240
[2465] Val Leu Lys Asp Tyr Leu Asn Gly Ile Ser Ser Glu Lys Pro Asn Gly
[2466] 245 250 255
[2467] Ile Tyr Ser Ile Tyr Asn Trp His Gln Leu Tyr His Ser Ser Tyr Tyr
[2468] 260 265 270
[2469] Phe Leu Arg Lys Asp Tyr Leu Thr Val Glu Thr Lys Leu His Asp Leu
[2470] 275 280 285
[2471] Arg Glu Tyr Leu Gly Gly Ser Leu Lys Gln Met Ser Trp Asp Thr Phe
[2472] 290 295 300
[2473] Ser Gln Leu Ser Lys Gly Asp Lys Glu Leu Phe Leu Asn Ile Val Gly
[2474] 305 310 315 320
[2475] Phe Asp Gln Glu Lys Leu Gln Gln Glu Tyr Gln Gln Ser Glu Leu Pro
[2476] 325 330 335
[2477] Asn Phe Val Phe Thr Gly Thr Thr Thr Trp Ala Gly Gly Glu Thr Lys

[2478] 340 345 350
[2479] Glu Tyr Tyr Ala Gln Gln Gln Val Asn Val Val Asn Asn Ala Ile Asn
[2480] 355 360 365
[2481] Glu Thr Ser Pro Tyr Tyr Leu Gly Arg Glu His Asp Leu Phe Phe Lys
[2482] 370 375 380
[2483] Gly His Pro Arg Gly Gly Ile Ile Asn Asp Ile Ile Leu Gly Ser Phe
[2484] 385 390 395 400
[2485] Asn Asn Met Ile Asp Ile Pro Ala Lys Val Ser Phe Glu Val Leu Met
[2486] 405 410 415
[2487] Met Thr Gly Met Leu Pro Asp Thr Val Gly Gly Ile Ala Ser Ser Leu
[2488] 420 425 430
[2489] Tyr Phe Ser Ile Pro Ala Glu Lys Val Ser Phe Ile Val Phe Thr Ser
[2490] 435 440 445
[2491] Ser Asp Thr Ile Thr Asp Arg Glu Asp Ala Leu Lys Ser Pro Leu Val
[2492] 450 455 460
[2493] Gln Val Met Met Thr Leu Gly Ile Val Lys Glu Lys Asp Val Leu Phe
[2494] 465 470 475 480
[2495] Trp Cys
[2496] <210> 64
[2497] <211> 675
[2498] <212> PRT
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[2500] <400> 64
[2501] Met Lys Lys Ile Leu Thr Val Leu Ser Ile Phe Ile Leu Ser Ala Cys
[2502] 1 5 10 15
[2503] Asn Ser Asp Asn Thr Ser Leu Lys Glu Thr Val Ser Ser Asn Ser Ala
[2504] 20 25 30
[2505] Asp Val Val Glu Thr Glu Thr Tyr Gln Leu Thr Pro Ile Asp Ala Pro
[2506] 35 40 45
[2507] Ser Ser Phe Leu Ser His Ser Trp Glu Gln Thr Cys Gly Thr Pro Ile
[2508] 50 55 60
[2509] Leu Asn Glu Ser Asp Lys Gln Ala Ile Ser Phe Asp Phe Val Ala Pro
[2510] 65 70 75 80
[2511] Glu Leu Lys Gln Asp Glu Lys Tyr Cys Phe Thr Phe Lys Gly Ile Thr
[2512] 85 90 95
[2513] Gly Asp His Arg Tyr Ile Thr Asn Thr Thr Leu Thr Val Val Ala Pro
[2514] 100 105 110
[2515] Thr Leu Glu Val Tyr Ile Asp His Ala Ser Leu Pro Ser Leu Gln Gln
[2516] 115 120 125
[2517] Leu Ile His Ile Ile Gln Ala Lys Asp Glu Tyr Pro Ser Asn Gln Arg
[2518] 130 135 140
[2519] Phe Val Ser Trp Lys Arg Val Thr Val Asp Ala Asp Asn Ala Asn Lys

[2520] 145 150 155 160
[2521] Leu Asn Ile His Thr Tyr Pro Leu Lys Gly Asn Asn Thr Ser Pro Glu
[2522] 165 170 175
[2523] Met Val Ala Ala Ile Asp Glu Tyr Ala Gln Ser Lys Asn Arg Leu Asn
[2524] 180 185 190
[2525] Ile Glu Phe Tyr Thr Asn Thr Ala His Val Phe Asn Asn Leu Pro Pro
[2526] 195 200 205
[2527] Ile Ile Gln Pro Leu Tyr Asn Asn Glu Lys Val Lys Ile Ser His Ile
[2528] 210 215 220
[2529] Ser Leu Tyr Asp Asp Gly Ser Ser Glu Tyr Val Ser Leu Tyr Gln Trp
[2530] 225 230 235 240
[2531] Lys Asp Thr Pro Asn Lys Ile Glu Thr Leu Glu Gly Glu Val Ser Leu
[2532] 245 250 255
[2533] Leu Ala Asn Tyr Leu Ala Gly Thr Ser Pro Asp Ala Pro Lys Gly Met
[2534] 260 265 270
[2535] Gly Asn Arg Tyr Asn Trp His Lys Leu Tyr Asp Thr Asp Tyr Tyr Phe
[2536] 275 280 285
[2537] Leu Arg Glu Asp Tyr Leu Asp Val Glu Ala Asn Leu His Asp Leu Arg
[2538] 290 295 300
[2539] Asp Tyr Leu Gly Ser Ser Ala Lys Gln Met Pro Trp Asp Glu Phe Ala
[2540] 305 310 315 320
[2541] Lys Leu Ser Asp Ser Gln Gln Thr Leu Phe Leu Asp Ile Val Gly Phe
[2542] 325 330 335
[2543] Asp Lys Glu Gln Leu Gln Gln Gln Tyr Ser Gln Ser Pro Leu Pro Asn
[2544] 340 345 350
[2545] Phe Ile Phe Thr Gly Thr Thr Thr Trp Ala Gly Gly Glu Thr Lys Glu
[2546] 355 360 365
[2547] Tyr Tyr Ala Gln Gln Gln Val Asn Val Ile Asn Asn Ala Ile Asn Glu
[2548] 370 375 380
[2549] Thr Ser Pro Tyr Tyr Leu Gly Lys Asp Tyr Asp Leu Phe Phe Lys Gly
[2550] 385 390 395 400
[2551] His Pro Ala Gly Gly Val Ile Asn Asp Ile Ile Leu Gly Ser Phe Pro
[2552] 405 410 415
[2553] Asp Met Ile Asn Ile Pro Ala Lys Ile Ser Phe Glu Val Leu Met Met
[2554] 420 425 430
[2555] Thr Asp Met Leu Pro Asp Thr Val Ala Gly Ile Ala Ser Ser Leu Tyr
[2556] 435 440 445
[2557] Phe Thr Ile Pro Ala Asp Lys Val Asn Phe Ile Val Phe Thr Ser Ser
[2558] 450 455 460
[2559] Asp Thr Ile Thr Asp Arg Glu Glu Ala Leu Lys Ser Pro Leu Val Gln
[2560] 465 470 475 480
[2561] Val Met Leu Thr Leu Gly Ile Val Lys Glu Lys Asp Val Leu Phe Trp

[2562] 485 490 495
[2563] Ala Asp His Lys Val Asn Ser Met Glu Val Ala Ile Asp Glu Ala Cys
[2564] 500 505 510
[2565] Thr Arg Ile Ile Ala Lys Arg Gln Pro Thr Ala Ser Asp Leu Arg Leu
[2566] 515 520 525
[2567] Val Ile Ala Ile Ile Lys Thr Ile Thr Asp Leu Glu Arg Ile Gly Asp
[2568] 530 535 540
[2569] Val Ala Glu Ser Ile Ala Lys Val Ala Leu Glu Ser Phe Ser Asn Lys
[2570] 545 550 555 560
[2571] Gln Tyr Asn Leu Leu Val Ser Leu Glu Ser Leu Gly Gln His Thr Val
[2572] 565 570 575
[2573] Arg Met Leu His Glu Val Leu Asp Ala Phe Ala Arg Met Asp Val Lys
[2574] 580 585 590
[2575] Ala Ala Ile Glu Val Tyr Gln Glu Asp Asp Arg Ile Asp Gln Glu Tyr
[2576] 595 600 605
[2577] Glu Ser Ile Val Arg Gln Leu Met Ala His Met Met Glu Asp Pro Ser
[2578] 610 615 620
[2579] Ser Ile Pro Asn Val Met Lys Val Met Trp Ala Ala Arg Ser Ile Glu
[2580] 625 630 635 640
[2581] Arg Val Gly Asp Arg Cys Gln Asn Ile Cys Glu Tyr Ile Ile Tyr Phe
[2582] 645 650 655
[2583] Val Lys Gly Lys Asp Val Arg His Thr Lys Pro Asp Asp Phe Gly Thr
[2584] 660 665 670
[2585] Met Leu Asp
[2586] 675
[2587] <210> 65
[2588] <211> 510
[2589] <212> PRT
[2590] <213> 美人鱼发光杆菌(Photobacterium damsela)
[2591] <400> 65
[2592] Met Lys Lys Ile Leu Thr Val Leu Ser Ile Phe Ile Leu Ser Ala Cys
[2593] 1 5 10 15
[2594] Asn Ser Asp Asn Thr Ser Leu Lys Glu Thr Val Ser Ser Asn Ser Ala
[2595] 20 25 30
[2596] Asp Val Val Glu Thr Glu Thr Tyr Gln Leu Thr Pro Ile Asp Ala Pro
[2597] 35 40 45
[2598] Ser Ser Phe Leu Ser His Ser Trp Glu Gln Thr Cys Gly Thr Pro Ile
[2599] 50 55 60
[2600] Leu Asn Glu Ser Asp Lys Gln Ala Ile Ser Phe Asp Phe Val Ala Pro
[2601] 65 70 75 80
[2602] Glu Leu Lys Gln Asp Glu Lys Tyr Cys Phe Thr Phe Lys Gly Ile Thr
[2603] 85 90 95

[2604] Gly Asp His Arg Tyr Ile Thr Asn Thr Thr Leu Thr Val Val Ala Pro
[2605] 100 105 110
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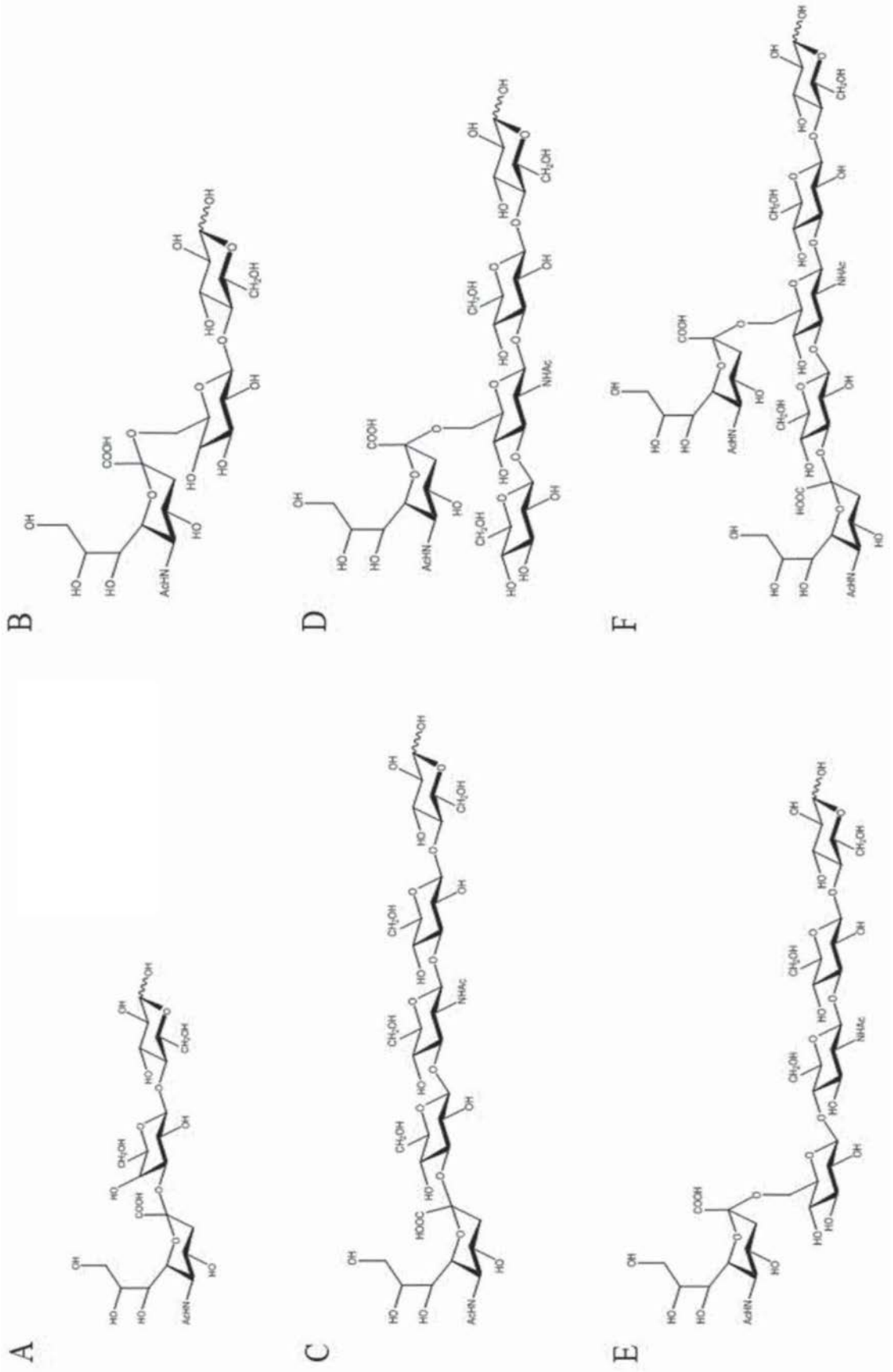



图1

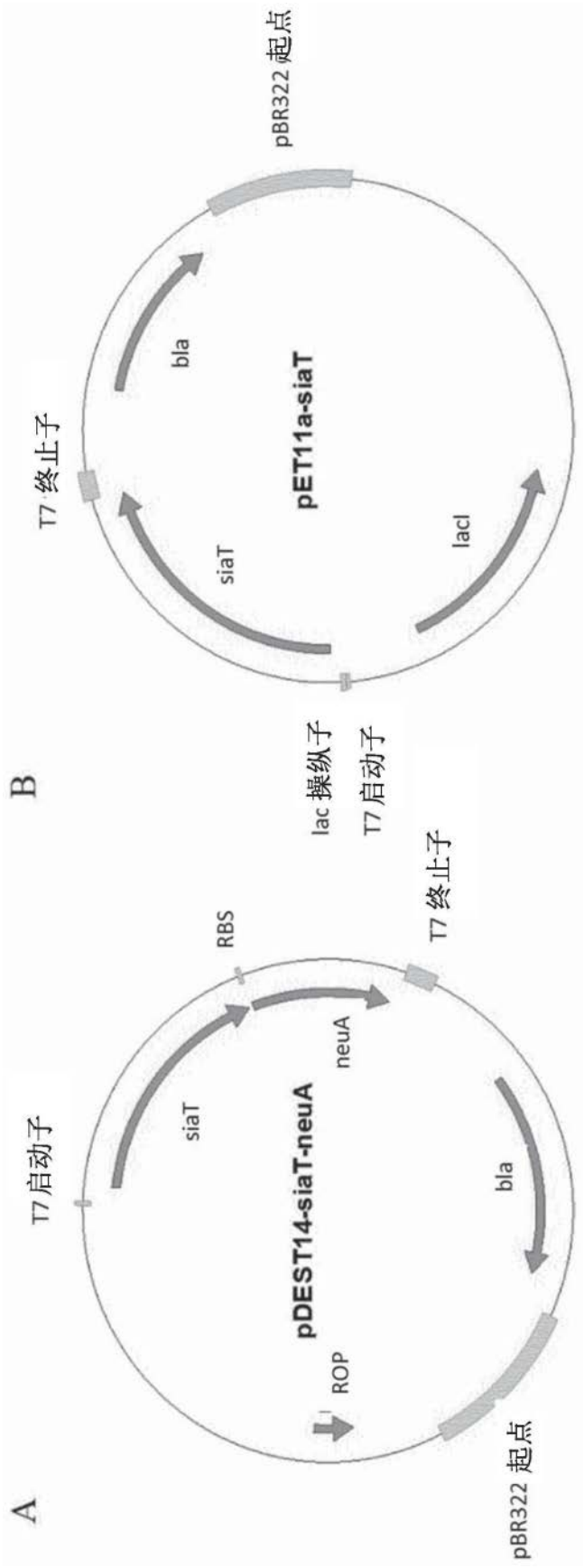

A
T7 启动子
siaT
RBS
neuA
pDEST14-siaT-neuA
ROP
T7 终止子
bla
pBR322 起点
B
lac 操纵子
T7 启动子
T7 终止子
pBR322 起点
bla
pET11a-siaT
lacI
siaT

图2

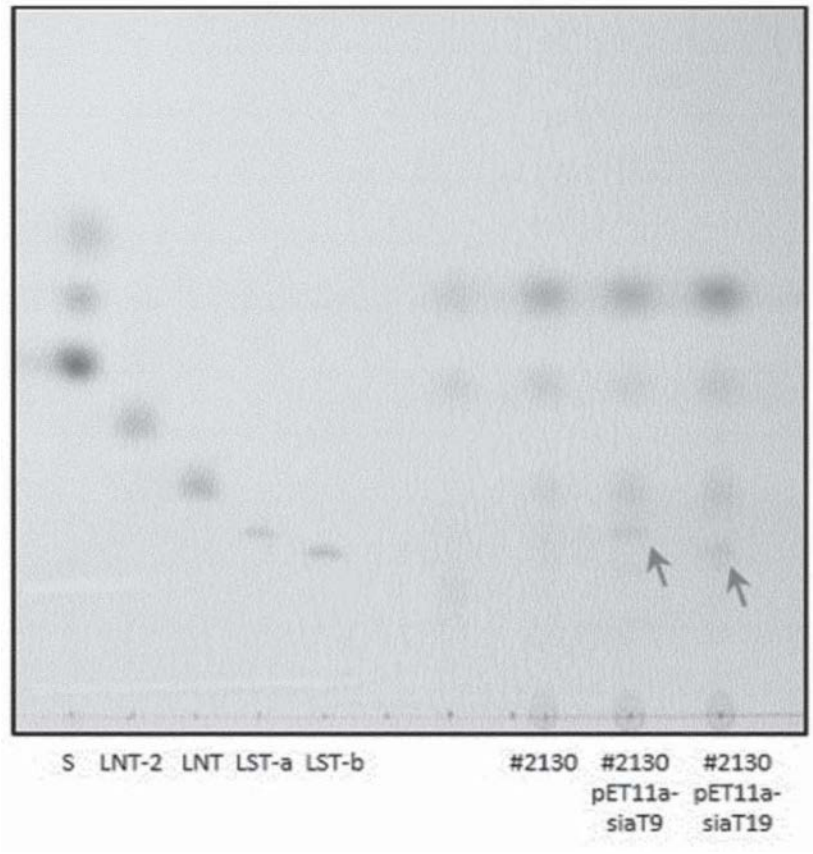

S
LNT-2
LNT
LST-a
LST-b
#2130
#2130 pET11a-siaT9
#2130 pET11a-siaT19

图3