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Huang等. Characterization of a new

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Flavobacterium sp. S20. 《J Ind Microbiol

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(54) 发明名称

海藻酸裂解酶突变体

(57) 摘要

本发明涉及蛋白质工程改造技术领域,具体涉及一种海藻酸裂解酶突变体。本发明通过随机突变的方法筛选获得的海藻酸裂解酶突变体,其在枯草芽孢杆菌中的表达水平得到显著提高。重组表达野生型海藻酸裂解酶的枯草芽孢杆菌,其摇瓶发酵上清液酶活为488 U/ml,而重组表达海藻酸裂解酶突变体的枯草芽孢杆菌,其摇瓶发酵上清液高达635-780 U/ml,提高了30%-60%,取得了意料不到的技术效果。所述突变体的应用将有利于降低海藻酸裂解酶的生产成本,市场前景广阔。

1. 一种海藻酸裂解酶突变体,其特征在于,所述的突变体的氨基酸序列如SEQ ID NO:3所示。
2. 编码权利要求1所述的海藻酸裂解酶突变体的DNA分子。
3. 一种重组表达载体,其特征在于,所述的重组表达载体携带有权利要求2所述的DNA分子。
4. 一种枯草芽孢杆菌 (*Bacillus subtilis*),其特征在于,所述的枯草芽孢杆菌包含有权利要求3所述的重组表达载体。
5. 权利要求4所述的枯草芽孢杆菌在生产海藻酸裂解酶中的应用。

海藻酸裂解酶突变体

技术领域

[0001] 本发明涉及蛋白质工程改造技术领域,具体涉及一种海藻酸裂解酶突变体及其应用。

背景技术

[0002] 海藻酸是储量仅次于纤维素的海洋生物多糖。海藻酸存在于海藻的细胞壁和细胞间质中,在褐藻中的含量最为丰富,大多数巨型褐藻是海藻酸的潜在来源。但不同种类的褐藻,其所含海藻酸的性质不同,所以要根据褐藻的可用性及其海藻酸的性质来选择褐藻的来源。海藻酸主要的商业来源是泡叶藻、公牛藻、翅藻、海带、巨藻、马尾藻和喇叭藻,其中最重要的是海带、巨藻和泡叶藻。全球海藻酸的产地主要分布于沿海国家和地区,如挪威、美国、法国、中国、日本和韩国。

[0003] 目前,海藻酸降解的方法可分为三大类:一类是化学降解法,目前广泛采用的是碱水解法,这种方法存在污染环境且能耗较大的不足,同时碱水解对海藻中的有效物质有所破坏。此外,还有过氧化氢氧化降解法;第二类是物理降解法,例如超声降解海藻酸;第三类是海藻酸裂解酶酶解法,酶法降解海藻酸条件温和,过程可控,得率高,绿色安全,环境友好,作用机理明确,产物确定,可以根据具体目的产物要求选择单一或组合使用不同底物专一性的酶制剂。

[0004] 海藻酸裂解酶通过 β 消去反应裂解海藻酸的4-O-糖基键,同时在C-4和C-5之间形成双键,在产生的寡糖的非还原端产生4-deoxy-L-erythro-hex-4-ene pyranosyluronate在230-240nm有强吸收峰。按底物的特异性,海藻酸裂解酶分为三种:第一种是聚甘露糖醛酸裂解酶,对polyM有特异性;第二种是聚古罗糖醛酸裂解酶,对polyG有特异性;第三种是对polyMG有特异性的裂解酶。按作用方式又可分为内切海藻酸裂解酶和外切海藻酸裂解酶。

[0005] 海藻酸裂解酶的来源广泛,主要有三大类,第一类是微生物,如海洋细菌、土壤细菌、真菌等;第二类是海洋软体动物和棘皮动物,如海螺、海参、鲍鱼等;第三类是植物,如巨藻、泡叶藻、海带等。

[0006] 目前,海藻酸裂解酶的生产大多是依靠海藻酸分解菌。虽然野生型海藻酸分解菌能有效的获得定量的酶蛋白,但产量很低成本较高,难达到实际应用要求。因此,利用基因工程手段对海藻酸裂解酶基因进行异源表达是提高海藻酸裂解酶产量的最有效途径。研究主要集中在海藻酸分解菌的海藻酸裂解酶基因的克隆和在大肠杆菌中过表达。目前,已有二十多种海藻酸分解菌的海藻酸裂解酶基因被克隆,而且其中大部分基因已成功地进行了异源表达。重组海藻酸裂解酶的表达量均高于野生菌株。1993年,Maki等研究人员,在大肠杆菌中表达了Pseudomonas sp. 0S-AIG-9的海藻酸裂解酶,其重组酶活性是野生菌株的53倍。

[0007] 近年来,随着蛋白质工程改造技术在酶制剂领域的广泛应用,新型的酶活水平高、性质优良的海藻酸裂解酶的开发成为本领域的研究热点,对于降低海藻酸裂解酶的生产成

本,促进海藻酸裂解酶的产业化具有重要的意义。

发明内容

[0008] 本发明为解决现有技术问题,提供了一种新型的海藻酸裂解酶突变体,并构建得到重组表达该突变体的枯草芽孢杆菌工程菌株。与野生型海藻酸裂解酶相比,所述海藻酸裂解酶突变体在枯草芽孢杆菌中的表达水平得到明显提高,有利于大幅降低该酶的生产成本,市场前景广阔。

[0009] 为了实现上述发明目的,本发明提供如下技术方案:

[0010] 本发明提供了一种海藻酸裂解酶突变体,其具有(I)、(II)或(III)所示的氨基酸序列中任意一个:

[0011] (I) 与海藻酸裂解酶的氨基酸序列SEQ ID NO:1具有至少95%同源性的序列;

[0012] (II) 具有所述(I)中所述的海藻酸裂解酶的至少一个免疫表位,且所述海藻酸裂解酶的氨基酸序列经修饰、取代、缺失或添加一个或几个氨基酸获得的氨基酸序列;

[0013] (III)由如SEQ ID NO:2所示的核苷酸序列或其互补序列或因遗传密码的简并性而与如SEQ ID NO:2所示的核苷酸序列或其互补序列的核苷酸序列不同的序列编码的氨基酸序列;

[0014] 在本发明的另一些实施例中,所述取代为取代1个氨基酸。

[0015] 在本发明的另一些实施例中,所述取代包括氨基酸序列为SEQ ID NO:1的海藻酸裂解酶的第33,49,50,53,79,133,188,196,203,205,239,243,271位氨基酸中任意一个发生了取代。

[0016] 在本发明的另一些实施例中,所述取代包括第33位氨基酸由K变N。

[0017] 在本发明的另一些实施例中,所述取代包括第49位氨基酸由D变为N。

[0018] 在本发明的另一些实施例中,所述取代包括第50位氨基酸由K变为G。

[0019] 在本发明的另一些实施例中,所述取代包括第53位氨基酸由K变为R。

[0020] 在本发明的另一些实施例中,所述取代包括第79位氨基酸由P变为D。

[0021] 在本发明的另一些实施例中,所述取代包括第133位氨基酸由D变为N。

[0022] 在本发明的另一些实施例中,所述取代包括第188位氨基酸由K变为D。

[0023] 在本发明的另一些实施例中,所述取代包括第196位氨基酸由P变为S。

[0024] 在本发明的另一些实施例中,所述取代包括第203位氨基酸由E变为S。

[0025] 在本发明的另一些实施例中,所述取代包括第205位氨基酸由A变为S。

[0026] 在本发明的另一些实施例中,所述取代包括第239位氨基酸由L变为I。

[0027] 在本发明的另一些实施例中,所述取代包括第243位氨基酸由E变为K。

[0028] 在本发明的另一些实施例中,所述取代包括第271位氨基酸由K变为N。

[0029] 在本发明的另一些实施例中,所述海藻酸裂解酶突变体的氨基酸序列如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:11,SEQ ID NO:12,SEQ ID NO:13,SEQ ID NO:14,SEQ ID NO:15所示。

[0030] 本发明还提供了编码上述氨基酸序列的DNA分子。

[0031] 本发明还提供了具有上述DNA分子的重组表达载体。

[0032] 本发明还提供了一种枯草芽孢杆菌(*Bacillus subtilis*),包含上述重组表达载体。

[0033] 本发明还提供了所述枯草芽孢杆菌在生产海藻酸裂解酶中的应用。

[0034] 与野生型海藻酸裂解酶相比,本发明通过随机突变的方法筛选获得的海藻酸裂解酶突变体,其在枯草芽孢杆菌中的表达水平得到显著提高。重组表达野生型海藻酸裂解酶的枯草芽孢杆菌,其摇瓶发酵上清液酶活为488 U/ml,而重组表达海藻酸裂解酶突变体的枯草芽孢杆菌,其摇瓶发酵上清液高达635-780 U/ml,提高了30%-60%,取得了意料不到的技术效果。所述突变体在枯草芽孢杆菌中的高效表达将有利于降低海藻酸裂解酶的生产成本,市场前景广阔。

具体实施方式

[0035] 本发明用到了遗传工程和分子生物学领域使用的常规技术和方法,例如 MOLECULAR CLONING: A LABORATORY MANUAL, 3rd Ed. (Sambrook, 2001) 和 CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Ausubel, 2003) 中所记载的方法。这些一般性参考文献提供了本领域技术人员已知的定义和方法。但是,本领域的技术人员可以在本发明所记载的技术方案的基础上,采用本领域其它常规的方法、实验方案和试剂,而限于本发明具体实施例的限定。

[0036] 本发明实施例中所述培养基中各组份及其含量分别为:

[0037] LB平板:胰蛋白胨 1%,酵母粉 0.5%,NaCl 1%,琼脂1.5%;

[0038] LB液体培养基:胰蛋白胨 1%,酵母粉 0.5%,NaCl 1%;

[0039] 1*最低盐溶液: K_2HPO_4 14 g/L, KH_2PO_4 6 g/L, $(NH_4)_2 SO_4$ 2 g/L,柠檬酸三钠 1 g/L, $MgSO_4 \cdot 7H_2O$ 0.2 g/L;

[0040] GM I溶液:1*最低盐溶液 95.6 ml,20%葡萄糖 2.5 ml,5%水解酪蛋白 0.4 ml,10% 酵母粉汁 1 ml;

[0041] GM II溶液:1*最低盐溶液96.98 ml,20%葡萄糖2.5 ml,5%水解酪蛋白0.08 ml,10%酵母粉汁0.04 ml,1 M $MgCl_2$ 0.25 ml,1 M $CaCl_2$ 0.05 ml;

[0042] 牛肉膏蛋白胨培养基:牛肉膏0.5%,蛋白胨1%,NaCl 0.5%,琼脂1.5%,pH 7.2。

[0043] 实施例1 海藻酸裂解酶突变体的筛选

[0044] 为了提高野生型海藻酸裂解酶AL(氨基酸序列为SEQ ID NO: 1,编码核苷酸序列为SEQ ID NO:2,由上海生工生物工程股份有限公司合成)的酶活力,申请人通过定向进化技术对该酶进行了大量突变的筛选,设计PCR引物AL-F1、AL-R1如下:

[0045] AL-F1:ggcgttcagcaacatgagcgcgcaggctcaggataaaaaatctaaatct;

[0046] AL-R1:ccgtcctctgttaacctcgagttattattaatgcgtcacctgaagacta;

[0047] 以野生型海藻酸裂解酶AL基因SEQ ID NO: 2为模板,利用上述引物用GeneMorph II随机突变PCR试剂盒(Stratagene)进行PCR扩增,胶回收PCR产物,连接到pSZX302质粒,转化至大肠杆菌BL21 (DE3)中,涂布于LB+Amp平板,37°C倒置培养,待转化子出现后,用牙签逐个挑至96孔板,每个孔中加入150 μ L含有0.1 mM IPTG的LB+Amp培养基,37°C,220rpm培养6 h左右,离心弃上清,菌体用缓冲液重悬,反复冻融破壁,获得含有海藻酸裂解酶的大肠杆菌细胞裂解液。

[0048] 各取50 μL裂解液至两块新的96孔板,分别在pH7.5的条件下测定其海藻酸裂解酶酶活。结果发现,与野生型海藻酸裂解酶AL相比,有些突变体在pH7.5条件下的酶活没有发生变化,有些突变体的酶活甚至降低了。最终,申请人筛选到能显著提高在pH7.5的条件下海藻酸裂解酶活性的突变位点:K33N,D49N,K50G,K53R,P79D,D133N,K188D,P196S,E203S,A205S,L239I,E243K和K271N。

[0049] 将含K33N单点突变的海藻酸裂解酶突变体命名为ALm1,其氨基酸序列为SEQ ID NO:3;

[0050] 将含D49N单点突变的海藻酸裂解酶突变体命名为ALm2,其氨基酸序列为SEQ ID NO:4;

[0051] 将含K50G单点突变的海藻酸裂解酶突变体命名为ALm3,其氨基酸序列为SEQ ID NO:5;

[0052] 将含K53R单点突变的海藻酸裂解酶突变体命名为AL-m4,其氨基酸序列为SEQ ID NO:6;

[0053] 将含P79D单点突变的海藻酸裂解酶突变体命名为ALm5,其氨基酸序列为SEQ ID NO:7;

[0054] 将含D133N单点突变的海藻酸裂解酶突变体命名为ALm6,其氨基酸序列为SEQ ID NO:8;

[0055] 将含K188D单点突变的海藻酸裂解酶突变体命名为ALm7,其氨基酸序列为SEQ ID NO:9;

[0056] 将含P196S单点突变的海藻酸裂解酶突变体命名为ALm8,其氨基酸序列为SEQ ID NO:10;

[0057] 将含E203S单点突变的海藻酸裂解酶突变体命名为ALm9,其氨基酸序列为SEQ ID NO:11;

[0058] 将含A205S单点突变的海藻酸裂解酶突变体命名为ALm10,其氨基酸序列为SEQ ID NO:12;

[0059] 将含L239I单点突变的海藻酸裂解酶突变体命名为ALm11,其氨基酸序列为SEQ ID NO:13;

[0060] 将含E243K单点突变的海藻酸裂解酶突变体命名为ALm12,其氨基酸序列为SEQ ID NO:14;

[0061] 将含K271N单点突变的海藻酸裂解酶突变体命名为ALm13,其氨基酸序列为SEQ ID NO:15.

[0062] 由上海捷瑞生物公司分别合成上述13个海藻酸裂解酶突变体的编码基因片段。

[0063] 将上述合成的海藻酸裂解酶突变体基因片段分别连接到pSZX302质粒,获得重组质粒,并送至北京华大基因研究中心进行测序分析。测序结果证明没有发生扩增错误。

[0064] 使用质粒中量制备试剂盒(Axygen)从测序结果正确的大肠杆菌克隆中纯化质粒,获得携带上述海藻酸裂解酶突变体基因序列的重组表达质粒。

[0065] 采用上述同样方法扩增得到野生型海藻酸裂解酶AL的基因片段,并构建得到携带野生型海藻酸裂解酶AL基因序列的重组表达质粒。

[0066] 实施例2 转化与筛选

[0067] 2.1转化

[0068] 将上述重组表达质粒分别转化入宿主菌枯草芽孢杆菌F4 (*Bacillus subtilis* F4), 分别获得重组表达野生型海藻酸裂解酶AL和海藻酸裂解酶突变体ALm1, ALm2, …… , ALm13的枯草芽孢杆菌重组菌株。

[0069] 具体转化过程如下:将新鲜活化的枯草芽孢杆菌F4由LB平板接种到5 ml GMI溶液中, 30℃、125 rpm振荡培养过夜, 得到培养液A; 取2 ml培养液A转接到18 ml GMI溶液中, 37℃、250 rpm培养3.5 h, 得到培养液B; 取10 ml培养液B转接到90 ml GMII溶液中, 37℃、125 rpm培养 90 min 后, 得到培养液C; 5000 g、10 min 离心收集培养液C中的菌体, 用10 ml GMII溶液轻轻悬浮菌体, 悬浮后的菌体即为感受态细胞。然后在 0.5 ml 感受态细胞中加入适量实施例1扩增得到的海藻酸裂解酶DNA, 37℃、200 rpm 振荡培养30 min后涂LB平板(含5μg/mL氯霉素), 37℃培养过夜, 次日检查和验证转化子。

[0070] 摇瓶发酵筛选

[0071] 将实施例2.1所述枯草芽孢杆菌重组菌株分别接种于50mL液体发酵培养基(酵母浸粉0.5%, 胰蛋白胨0.5%, 葡萄糖1%, K_2HPO_4 1.8%), 摇瓶发酵48 h后, 5000 g、10 min 离心收集上清液, 分别测定上清液中海藻酸裂解酶酶活。

[0072] 海藻酸裂解酶酶活的测定方法如下:

[0073] 1、酶活力单位定义

[0074] 在40℃, pH为7.5条件下, 在本方法规定反应体系下, 每分钟降解底物海藻酸钠产生不饱和键, 在235nm下, 吸光度每增加0.1, 为一个酶活单位U。

[0075] 2、原理

[0076] 海藻酸裂解酶可以通过β-消除反应切断海藻酸分子中的糖苷键, 产生非还原性端具有不饱和双键, 双键位于产物非还原末端C₄、C₅之间, 并在235nm处产生最大紫外吸收。

[0077] 3、测定方法

[0078] 3.1 液体样品: 用缓冲液稀释至适当倍数, 控制在吸光值OD₂₃₅在0.22-0.35之间, 酶活约为0.5 U/mL。

[0079] 3.2 测定步骤

[0080] 酶反应: 取三支15mm*150mm试管, 加入1.8mL底物, 40℃水浴预热5min, 加入稀释好的酶液0.2mL, 准确计时, 涡旋震荡, 40℃保温10min, 将试管从水浴中取出并立即加入2mL磷酸终止液, 涡旋振荡, 将试管放置在水浴锅外的试管架上。

[0081] 空白: 取15mm*150mm试管, 加入1.8mL底物, 40℃水浴预热5min, 加入缓冲液0.2mL, 涡旋震荡, 40℃保温10min, 将试管从水浴中取出并立即加入2mL磷酸终止液, 涡旋振荡, 将试管放置在水浴锅外的试管架上。

[0082] 比色: 每个样品的空白和酶反应终止后, 立即在235nm比色, 并记录吸光值A₀和A_样。

[0083] 备注:

[0084] 8. 计算

[0085] $X = (A_0 - A_{\text{样}}) \times 2 \times N / (t \times 0.1)$

[0086] 式中:

[0087] X— 酶活力, U/mL或U/g

[0088] 2—加入2mL磷酸终止液的体积系数

[0089] t (min)—酶促反应时间(在酶反应的线性范围内)

[0090] 0.1—体系系数,即将吸光值增长单位换算为0.1

[0091] N—稀释倍数

[0092] 经简化:酶活力(U/mL) = $(A_0 - A_{\text{样}}) \times 2 \times N$ 。

[0093] 下面结合具体实施方式对本发明进行详细描述。

[0094] 酶活测定结果显示,重组表达野生型海藻酸裂解酶的枯草芽孢杆菌,其发酵上清液酶活为488 U/ml,而重组表达海藻酸裂解酶突变体的枯草芽孢杆菌,其发酵上清液高达635-780 U/ml,提高了30%-60%。从而表明本发明提供的海藻酸裂解酶突变体ALm1, ALm2, …… ,ALm13在枯草芽孢杆菌中的表达水平,要显著高于野生型海藻酸裂解酶AL,取得了意料不到的技术效果。

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 [0002] <110> 青岛蔚蓝生物集团有限公司
 [0003] 潍坊康地恩生物科技有限公司
 [0004] <120> 海藻酸裂解酶突变体
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| [0063] | ttactggaac atgcgtgggg agatgatgaa ggacgtaatt taaagaaaa aatcgatttg | 660 | | |
| [0064] | aatacacgct ttacgcttga agtcaaagtc tctgatggcc gcatggaagt catccttaat | 720 | | |
| [0065] | gatacagaat cacttgtgta tgatgacata cacatgaaaa aatggggcat ctttgaaaaat | 780 | | |
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| [0081] | Tyr Ala Glu Asp Lys Ile Ala Ser Lys Tyr Met Tyr Asp Asp Pro Lys | | |
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| [0083] | Asp Lys Ser Val Val Phe Tyr Ala Phe Pro Ser Gly Val Thr Thr Ala | | |
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 [0292] Ser Asn Lys Val Asn Trp Thr Phe Ala Lys Gly Gly Lys Met Arg Gly
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 [0296] Arg Val Ile Ile Ala Gln Ile His Gly Val Leu Thr Asp Glu Gln Arg
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 [0307] 225 230 235 240
 [0308] Asp Thr Glu Ser Leu Val Tyr Asp Asp Ile His Met Lys Lys Trp Gly
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 [0310] Ile Phe Glu Asn Tyr Phe Lys Ala Gly Asn Tyr Phe Gln Ser Lys Thr
 [0311] 260 265 270

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| [0324] | 35 40 45 |
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| [0327] | Tyr Ala Glu Asp Lys Ile Ala Ser Lys Tyr Met Tyr Asp Asp Pro Lys |
| [0328] | 65 70 75 80 |
| [0329] | Asp Lys Ser Val Val Phe Tyr Ala Phe Pro Ser Gly Val Thr Thr Ala |
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| [0331] | Asn Thr His Tyr Ser Arg Ser Glu Leu Arg Glu Thr Met Glu Thr Gly |
| [0332] | 100 105 110 |
| [0333] | Ser Asn Lys Val Asn Trp Thr Phe Ala Lys Gly Gly Lys Met Arg Gly |
| [0334] | 115 120 125 |
| [0335] | Thr Tyr Ala Ile Asp Asp Ile Ser Lys Glu Pro Asp Gly Lys Tyr Ser |
| [0336] | 130 135 140 |
| [0337] | Arg Val Ile Ile Ala Gln Ile His Gly Val Leu Thr Asp Glu Gln Arg |
| [0338] | 145 150 155 160 |
| [0339] | Asp Leu Ile Gly Gln Lys Asp Asn Asn Ala Pro Pro Ile Leu Lys Val |
| [0340] | 165 170 175 |
| [0341] | Tyr Trp Asp Lys Gly Lys Ile Arg Val Lys Thr Asp Val Leu Lys Asp |
| [0342] | 180 185 190 |
| [0343] | Leu Asn Ala Pro Tyr Lys Glu Met Leu Leu Glu His Ala Trp Gly Asp |
| [0344] | 195 200 205 |
| [0345] | Asp Glu Gly Arg Asn Phe Lys Glu Lys Ile Asp Leu Asn Thr Arg Phe |
| [0346] | 210 215 220 |
| [0347] | Thr Leu Glu Val Lys Val Ser Asp Gly Arg Met Glu Val Ile Leu Asn |
| [0348] | 225 230 235 240 |
| [0349] | Asp Thr Glu Ser Leu Val Tyr Asp Asp Ile His Met Lys Lys Trp Gly |
| [0350] | 245 250 255 |

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|--------|---|
| [0351] | Ile Phe Glu Asn Tyr Phe Lys Ala Gly Asn Tyr Phe Gln Ser Lys Thr |
| [0352] | 260 265 270 |
| [0353] | Pro Gly Thr Phe Ala Lys Val Lys Ile Tyr Ser Leu Gln Val Thr His |
| [0354] | 275 280 285 |
| [0355] | <210> 10 |
| [0356] | <211> 288 |
| [0357] | <212> PRT |
| [0358] | <213> 人工序列(Artificial Sequence) |
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| [0360] | Met Ser Ile Gln Phe Ser Lys Ile Leu Leu Leu Thr Val Leu Ala Thr |
| [0361] | 1 5 10 15 |
| [0362] | Ala Thr Ile Ser Asn Ala Gln Asp Lys Lys Ser Lys Ser Lys Thr Ala |
| [0363] | 20 25 30 |
| [0364] | Lys Ile Asp Trp Ser His Trp Thr Val Thr Val Pro Glu Glu Asn Pro |
| [0365] | 35 40 45 |
| [0366] | Asp Lys Pro Gly Lys Pro Tyr Ser Leu Gly Tyr Pro Glu Ile Leu Asn |
| [0367] | 50 55 60 |
| [0368] | Tyr Ala Glu Asp Lys Ile Ala Ser Lys Tyr Met Tyr Asp Asp Pro Lys |
| [0369] | 65 70 75 80 |
| [0370] | Asp Lys Ser Val Val Phe Tyr Ala Phe Pro Ser Gly Val Thr Thr Ala |
| [0371] | 85 90 95 |
| [0372] | Asn Thr His Tyr Ser Arg Ser Glu Leu Arg Glu Thr Met Glu Thr Gly |
| [0373] | 100 105 110 |
| [0374] | Ser Asn Lys Val Asn Trp Thr Phe Ala Lys Gly Gly Lys Met Arg Gly |
| [0375] | 115 120 125 |
| [0376] | Thr Tyr Ala Ile Asp Asp Ile Ser Lys Glu Pro Asp Gly Lys Tyr Ser |
| [0377] | 130 135 140 |
| [0378] | Arg Val Ile Ile Ala Gln Ile His Gly Val Leu Thr Asp Glu Gln Arg |
| [0379] | 145 150 155 160 |
| [0380] | Asp Leu Ile Gly Gln Lys Asp Asn Asn Ala Pro Pro Ile Leu Lys Val |
| [0381] | 165 170 175 |
| [0382] | Tyr Trp Asp Lys Gly Lys Ile Arg Val Lys Thr Lys Val Leu Lys Asp |
| [0383] | 180 185 190 |
| [0384] | Leu Asn Ala Ser Tyr Lys Glu Met Leu Leu Glu His Ala Trp Gly Asp |
| [0385] | 195 200 205 |
| [0386] | Asp Glu Gly Arg Asn Phe Lys Glu Lys Ile Asp Leu Asn Thr Arg Phe |
| [0387] | 210 215 220 |
| [0388] | Thr Leu Glu Val Lys Val Ser Asp Gly Arg Met Glu Val Ile Leu Asn |
| [0389] | 225 230 235 240 |

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|--------|---|
| [0390] | Asp Thr Glu Ser Leu Val Tyr Asp Asp Ile His Met Lys Lys Trp Gly |
| [0391] | 245 250 255 |
| [0392] | Ile Phe Glu Asn Tyr Phe Lys Ala Gly Asn Tyr Phe Gln Ser Lys Thr |
| [0393] | 260 265 270 |
| [0394] | Pro Gly Thr Phe Ala Lys Val Lys Ile Tyr Ser Leu Gln Val Thr His |
| [0395] | 275 280 285 |
| [0396] | <210> 11 |
| [0397] | <211> 288 |
| [0398] | <212> PRT |
| [0399] | <213> 人工序列(Artificial Sequence) |
| [0400] | <400> 11 |
| [0401] | Met Ser Ile Gln Phe Ser Lys Ile Leu Leu Leu Thr Val Leu Ala Thr |
| [0402] | 1 5 10 15 |
| [0403] | Ala Thr Ile Ser Asn Ala Gln Asp Lys Lys Ser Lys Ser Lys Thr Ala |
| [0404] | 20 25 30 |
| [0405] | Lys Ile Asp Trp Ser His Trp Thr Val Thr Val Pro Glu Glu Asn Pro |
| [0406] | 35 40 45 |
| [0407] | Asp Lys Pro Gly Lys Pro Tyr Ser Leu Gly Tyr Pro Glu Ile Leu Asn |
| [0408] | 50 55 60 |
| [0409] | Tyr Ala Glu Asp Lys Ile Ala Ser Lys Tyr Met Tyr Asp Asp Pro Lys |
| [0410] | 65 70 75 80 |
| [0411] | Asp Lys Ser Val Val Phe Tyr Ala Phe Pro Ser Gly Val Thr Thr Ala |
| [0412] | 85 90 95 |
| [0413] | Asn Thr His Tyr Ser Arg Ser Glu Leu Arg Glu Thr Met Glu Thr Gly |
| [0414] | 100 105 110 |
| [0415] | Ser Asn Lys Val Asn Trp Thr Phe Ala Lys Gly Gly Lys Met Arg Gly |
| [0416] | 115 120 125 |
| [0417] | Thr Tyr Ala Ile Asp Asp Ile Ser Lys Glu Pro Asp Gly Lys Tyr Ser |
| [0418] | 130 135 140 |
| [0419] | Arg Val Ile Ile Ala Gln Ile His Gly Val Leu Thr Asp Glu Gln Arg |
| [0420] | 145 150 155 160 |
| [0421] | Asp Leu Ile Gly Gln Lys Asp Asn Asn Ala Pro Pro Ile Leu Lys Val |
| [0422] | 165 170 175 |
| [0423] | Tyr Trp Asp Lys Gly Lys Ile Arg Val Lys Thr Lys Val Leu Lys Asp |
| [0424] | 180 185 190 |
| [0425] | Leu Asn Ala Pro Tyr Lys Glu Met Leu Leu Ser His Ala Trp Gly Asp |
| [0426] | 195 200 205 |
| [0427] | Asp Glu Gly Arg Asn Phe Lys Glu Lys Ile Asp Leu Asn Thr Arg Phe |
| [0428] | 210 215 220 |

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|--------|---|
| [0429] | Thr Leu Glu Val Lys Val Ser Asp Gly Arg Met Glu Val Ile Leu Asn |
| [0430] | 225 230 235 240 |
| [0431] | Asp Thr Glu Ser Leu Val Tyr Asp Asp Ile His Met Lys Lys Trp Gly |
| [0432] | 245 250 255 |
| [0433] | Ile Phe Glu Asn Tyr Phe Lys Ala Gly Asn Tyr Phe Gln Ser Lys Thr |
| [0434] | 260 265 270 |
| [0435] | Pro Gly Thr Phe Ala Lys Val Lys Ile Tyr Ser Leu Gln Val Thr His |
| [0436] | 275 280 285 |
| [0437] | <210> 12 |
| [0438] | <211> 288 |
| [0439] | <212> PRT |
| [0440] | <213> 人工序列(Artificial Sequence) |
| [0441] | <400> 12 |
| [0442] | Met Ser Ile Gln Phe Ser Lys Ile Leu Leu Leu Thr Val Leu Ala Thr |
| [0443] | 1 5 10 15 |
| [0444] | Ala Thr Ile Ser Asn Ala Gln Asp Lys Lys Ser Lys Ser Lys Thr Ala |
| [0445] | 20 25 30 |
| [0446] | Lys Ile Asp Trp Ser His Trp Thr Val Thr Val Pro Glu Glu Asn Pro |
| [0447] | 35 40 45 |
| [0448] | Asp Lys Pro Gly Lys Pro Tyr Ser Leu Gly Tyr Pro Glu Ile Leu Asn |
| [0449] | 50 55 60 |
| [0450] | Tyr Ala Glu Asp Lys Ile Ala Ser Lys Tyr Met Tyr Asp Asp Pro Lys |
| [0451] | 65 70 75 80 |
| [0452] | Asp Lys Ser Val Val Phe Tyr Ala Phe Pro Ser Gly Val Thr Thr Ala |
| [0453] | 85 90 95 |
| [0454] | Asn Thr His Tyr Ser Arg Ser Glu Leu Arg Glu Thr Met Glu Thr Gly |
| [0455] | 100 105 110 |
| [0456] | Ser Asn Lys Val Asn Trp Thr Phe Ala Lys Gly Gly Lys Met Arg Gly |
| [0457] | 115 120 125 |
| [0458] | Thr Tyr Ala Ile Asp Asp Ile Ser Lys Glu Pro Asp Gly Lys Tyr Ser |
| [0459] | 130 135 140 |
| [0460] | Arg Val Ile Ile Ala Gln Ile His Gly Val Leu Thr Asp Glu Gln Arg |
| [0461] | 145 150 155 160 |
| [0462] | Asp Leu Ile Gly Gln Lys Asp Asn Asn Ala Pro Pro Ile Leu Lys Val |
| [0463] | 165 170 175 |
| [0464] | Tyr Trp Asp Lys Gly Lys Ile Arg Val Lys Thr Lys Val Leu Lys Asp |
| [0465] | 180 185 190 |
| [0466] | Leu Asn Ala Pro Tyr Lys Glu Met Leu Leu Glu His Ser Trp Gly Asp |
| [0467] | 195 200 205 |

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|--------|---|
| [0468] | Asp Glu Gly Arg Asn Phe Lys Glu Lys Ile Asp Leu Asn Thr Arg Phe |
| [0469] | 210 215 220 |
| [0470] | Thr Leu Glu Val Lys Val Ser Asp Gly Arg Met Glu Val Ile Leu Asn |
| [0471] | 225 230 235 240 |
| [0472] | Asp Thr Glu Ser Leu Val Tyr Asp Asp Ile His Met Lys Lys Trp Gly |
| [0473] | 245 250 255 |
| [0474] | Ile Phe Glu Asn Tyr Phe Lys Ala Gly Asn Tyr Phe Gln Ser Lys Thr |
| [0475] | 260 265 270 |
| [0476] | Pro Gly Thr Phe Ala Lys Val Lys Ile Tyr Ser Leu Gln Val Thr His |
| [0477] | 275 280 285 |
| [0478] | <210> 13 |
| [0479] | <211> 288 |
| [0480] | <212> PRT |
| [0481] | <213> 人工序列(Artificial Sequence) |
| [0482] | <400> 13 |
| [0483] | Met Ser Ile Gln Phe Ser Lys Ile Leu Leu Leu Thr Val Leu Ala Thr |
| [0484] | 1 5 10 15 |
| [0485] | Ala Thr Ile Ser Asn Ala Gln Asp Lys Lys Ser Lys Ser Lys Thr Ala |
| [0486] | 20 25 30 |
| [0487] | Lys Ile Asp Trp Ser His Trp Thr Val Thr Val Pro Glu Glu Asn Pro |
| [0488] | 35 40 45 |
| [0489] | Asp Lys Pro Gly Lys Pro Tyr Ser Leu Gly Tyr Pro Glu Ile Leu Asn |
| [0490] | 50 55 60 |
| [0491] | Tyr Ala Glu Asp Lys Ile Ala Ser Lys Tyr Met Tyr Asp Asp Pro Lys |
| [0492] | 65 70 75 80 |
| [0493] | Asp Lys Ser Val Val Phe Tyr Ala Phe Pro Ser Gly Val Thr Thr Ala |
| [0494] | 85 90 95 |
| [0495] | Asn Thr His Tyr Ser Arg Ser Glu Leu Arg Glu Thr Met Glu Thr Gly |
| [0496] | 100 105 110 |
| [0497] | Ser Asn Lys Val Asn Trp Thr Phe Ala Lys Gly Gly Lys Met Arg Gly |
| [0498] | 115 120 125 |
| [0499] | Thr Tyr Ala Ile Asp Asp Ile Ser Lys Glu Pro Asp Gly Lys Tyr Ser |
| [0500] | 130 135 140 |
| [0501] | Arg Val Ile Ile Ala Gln Ile His Gly Val Leu Thr Asp Glu Gln Arg |
| [0502] | 145 150 155 160 |
| [0503] | Asp Leu Ile Gly Gln Lys Asp Asn Asn Ala Pro Pro Ile Leu Lys Val |
| [0504] | 165 170 175 |
| [0505] | Tyr Trp Asp Lys Gly Lys Ile Arg Val Lys Thr Lys Val Leu Lys Asp |
| [0506] | 180 185 190 |

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|--------|---|
| [0507] | Leu Asn Ala Pro Tyr Lys Glu Met Leu Leu Glu His Ala Trp Gly Asp |
| [0508] | 195 200 205 |
| [0509] | Asp Glu Gly Arg Asn Phe Lys Glu Lys Ile Asp Leu Asn Thr Arg Phe |
| [0510] | 210 215 220 |
| [0511] | Thr Leu Glu Val Lys Val Ser Asp Gly Arg Met Glu Val Ile Ile Asn |
| [0512] | 225 230 235 240 |
| [0513] | Asp Thr Glu Ser Leu Val Tyr Asp Asp Ile His Met Lys Lys Trp Gly |
| [0514] | 245 250 255 |
| [0515] | Ile Phe Glu Asn Tyr Phe Lys Ala Gly Asn Tyr Phe Gln Ser Lys Thr |
| [0516] | 260 265 270 |
| [0517] | Pro Gly Thr Phe Ala Lys Val Lys Ile Tyr Ser Leu Gln Val Thr His |
| [0518] | 275 280 285 |
| [0519] | <210> 14 |
| [0520] | <211> 288 |
| [0521] | <212> PRT |
| [0522] | <213> 人工序列(Artificial Sequence) |
| [0523] | <400> 14 |
| [0524] | Met Ser Ile Gln Phe Ser Lys Ile Leu Leu Leu Thr Val Leu Ala Thr |
| [0525] | 1 5 10 15 |
| [0526] | Ala Thr Ile Ser Asn Ala Gln Asp Lys Lys Ser Lys Ser Lys Thr Ala |
| [0527] | 20 25 30 |
| [0528] | Lys Ile Asp Trp Ser His Trp Thr Val Thr Val Pro Glu Glu Asn Pro |
| [0529] | 35 40 45 |
| [0530] | Asp Lys Pro Gly Lys Pro Tyr Ser Leu Gly Tyr Pro Glu Ile Leu Asn |
| [0531] | 50 55 60 |
| [0532] | Tyr Ala Glu Asp Lys Ile Ala Ser Lys Tyr Met Tyr Asp Asp Pro Lys |
| [0533] | 65 70 75 80 |
| [0534] | Asp Lys Ser Val Val Phe Tyr Ala Phe Pro Ser Gly Val Thr Thr Ala |
| [0535] | 85 90 95 |
| [0536] | Asn Thr His Tyr Ser Arg Ser Glu Leu Arg Glu Thr Met Glu Thr Gly |
| [0537] | 100 105 110 |
| [0538] | Ser Asn Lys Val Asn Trp Thr Phe Ala Lys Gly Gly Lys Met Arg Gly |
| [0539] | 115 120 125 |
| [0540] | Thr Tyr Ala Ile Asp Asp Ile Ser Lys Glu Pro Asp Gly Lys Tyr Ser |
| [0541] | 130 135 140 |
| [0542] | Arg Val Ile Ile Ala Gln Ile His Gly Val Leu Thr Asp Glu Gln Arg |
| [0543] | 145 150 155 160 |
| [0544] | Asp Leu Ile Gly Gln Lys Asp Asn Asn Ala Pro Pro Ile Leu Lys Val |
| [0545] | 165 170 175 |

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| [0546] | Tyr Trp Asp Lys Gly Lys Ile Arg Val Lys Thr Lys Val Leu Lys Asp |
| [0547] | 180 185 190 |
| [0548] | Leu Asn Ala Pro Tyr Lys Glu Met Leu Leu Glu His Ala Trp Gly Asp |
| [0549] | 195 200 205 |
| [0550] | Asp Glu Gly Arg Asn Phe Lys Glu Lys Ile Asp Leu Asn Thr Arg Phe |
| [0551] | 210 215 220 |
| [0552] | Thr Leu Glu Val Lys Val Ser Asp Gly Arg Met Glu Val Ile Leu Asn |
| [0553] | 225 230 235 240 |
| [0554] | Asp Thr Lys Ser Leu Val Tyr Asp Asp Ile His Met Lys Lys Trp Gly |
| [0555] | 245 250 255 |
| [0556] | Ile Phe Glu Asn Tyr Phe Lys Ala Gly Asn Tyr Phe Gln Ser Lys Thr |
| [0557] | 260 265 270 |
| [0558] | Pro Gly Thr Phe Ala Lys Val Lys Ile Tyr Ser Leu Gln Val Thr His |
| [0559] | 275 280 285 |
| [0560] | <210> 15 |
| [0561] | <211> 288 |
| [0562] | <212> PRT |
| [0563] | <213> 人工序列(Artificial Sequence) |
| [0564] | <400> 15 |
| [0565] | Met Ser Ile Gln Phe Ser Lys Ile Leu Leu Leu Thr Val Leu Ala Thr |
| [0566] | 1 5 10 15 |
| [0567] | Ala Thr Ile Ser Asn Ala Gln Asp Lys Lys Ser Lys Ser Lys Thr Ala |
| [0568] | 20 25 30 |
| [0569] | Lys Ile Asp Trp Ser His Trp Thr Val Thr Val Pro Glu Glu Asn Pro |
| [0570] | 35 40 45 |
| [0571] | Asp Lys Pro Gly Lys Pro Tyr Ser Leu Gly Tyr Pro Glu Ile Leu Asn |
| [0572] | 50 55 60 |
| [0573] | Tyr Ala Glu Asp Lys Ile Ala Ser Lys Tyr Met Tyr Asp Asp Pro Lys |
| [0574] | 65 70 75 80 |
| [0575] | Asp Lys Ser Val Val Phe Tyr Ala Phe Pro Ser Gly Val Thr Thr Ala |
| [0576] | 85 90 95 |
| [0577] | Asn Thr His Tyr Ser Arg Ser Glu Leu Arg Glu Thr Met Glu Thr Gly |
| [0578] | 100 105 110 |
| [0579] | Ser Asn Lys Val Asn Trp Thr Phe Ala Lys Gly Gly Lys Met Arg Gly |
| [0580] | 115 120 125 |
| [0581] | Thr Tyr Ala Ile Asp Asp Ile Ser Lys Glu Pro Asp Gly Lys Tyr Ser |
| [0582] | 130 135 140 |
| [0583] | Arg Val Ile Ile Ala Gln Ile His Gly Val Leu Thr Asp Glu Gln Arg |
| [0584] | 145 150 155 160 |

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|--------|---|
| [0585] | Asp Leu Ile Gly Gln Lys Asp Asn Asn Ala Pro Pro Ile Leu Lys Val |
| [0586] | 165 170 175 |
| [0587] | Tyr Trp Asp Lys Gly Lys Ile Arg Val Lys Thr Lys Val Leu Lys Asp |
| [0588] | 180 185 190 |
| [0589] | Leu Asn Ala Pro Tyr Lys Glu Met Leu Leu Glu His Ala Trp Gly Asp |
| [0590] | 195 200 205 |
| [0591] | Asp Glu Gly Arg Asn Phe Lys Glu Lys Ile Asp Leu Asn Thr Arg Phe |
| [0592] | 210 215 220 |
| [0593] | Thr Leu Glu Val Lys Val Ser Asp Gly Arg Met Glu Val Ile Leu Asn |
| [0594] | 225 230 235 240 |
| [0595] | Asp Thr Glu Ser Leu Val Tyr Asp Asp Ile His Met Lys Lys Trp Gly |
| [0596] | 245 250 255 |
| [0597] | Ile Phe Glu Asn Tyr Phe Lys Ala Gly Asn Tyr Phe Gln Ser Asn Thr |
| [0598] | 260 265 270 |
| [0599] | Pro Gly Thr Phe Ala Lys Val Lys Ile Tyr Ser Leu Gln Val Thr His |
| [0600] | 275 280 285 |