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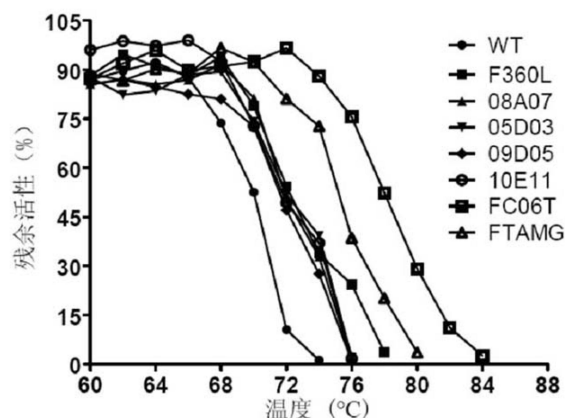
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(54) 发明名称

一种 1, 4- β -D- 木聚糖酶突变体

(57) 摘要

本发明属于基因工程和酶工程技术领域, 具体涉及 1, 4- β -D- 木聚糖酶突变体。本发明使用易错 PCR 方法、DNA 改组技术对该酶基因进行突变, 再通过高通量筛选方法将正突变检出。并结合基于序列比对的半理性设计方法确定部分潜在热稳定相关位点, 再通过定点突变方法得到热稳定相关突变体。经上述突变文库构建、筛选以及半理性设计方法, 获得 5 个热稳定性显著提高的突变体, 其热失活半衰期比野生型提高 2-52 倍, 显示出在造纸制浆、生物能源等工业上潜在的应用价值。



1. 一种热稳定性提高的 1,4- β -D-木聚糖酶突变体,其特征在于:以 SEQ ID NO.1 所示的 1,4- β -D-木聚糖酶 XT6 氨基酸序列为基础,将其第 271 位的蛋氨酸突变为异亮氨酸。

2. 一种热稳定性提高的 1,4- β -D-木聚糖酶突变体,其特征在于:将权利要求 1 所述的突变体的第 257 位的丙氨酸突变为缬氨酸。

3. 一种热稳定性提高的 1,4- β -D-木聚糖酶突变体,其特征在于:将权利要求 1 所述的突变体的第 257 位的丙氨酸突变为缬氨酸且第 364 位的甘氨酸突变为天冬氨酸。

4. 一种热稳定性提高的 1,4- β -D-木聚糖酶突变体,其特征在于:将权利要求 1 所述的突变体的第 213 位的苏氨酸突变为异亮氨酸、第 257 位的丙氨酸突变为亮氨酸且 364 位的甘氨酸突变为天冬氨酸。

5. 一种热稳定性提高的 1,4- β -D-木聚糖酶突变体,其特征在于:将权利要求 1 所述的突变体的第 138 位的谷氨酸突变为天冬氨酸、第 257 位的丙氨酸突变为缬氨酸且第 364 位的甘氨酸突变为天冬氨酸。

6. 如权利要求 1 或 2 或 3 或 4 或 5 所述的一种热稳定性提高的 1,4- β -D-木聚糖酶突变体,其特征在于,将突变体的基因连入 pET28a(+) 载体,并在宿主细胞大肠杆菌 *Escherichia coli* BL21-DE3 中表达,获得突变体酶蛋白。

一种 1, 4- β -D- 木聚糖酶突变体

技术领域

[0001] 本发明属于基因工程和酶工程领域, 具体内容涉及耐热性提高的 1, 4- β -D- 木聚糖酶突变体。

背景技术

[0002] 木聚糖酶 (endo-1, 4- β -xylanases, EC 3.2.1.8) 以内切方式水解木聚糖分子中的 β -1, 4- 糖苷键, 生成低聚木糖和木糖, 是半纤维素水解酶系中最关键的水解酶之一, 在工业上具有重要的应用价值。自上世纪 80 年代木聚糖酶开始工业应用以来, 木聚糖酶的应用领域不断扩大, 目前已经在饲料、制浆造纸、食品、能源等行业中得到广泛应用。随着木聚糖酶的应用范围进一步拓展, 工业上对其现有性能提出了更高的要求, 如长时间保持活性稳定, 在极端环境中保持高的活性 (极端温度或者 pH 值等) 或者可以接受不同的底物 (包括非天然底物)。其中酶的热稳定性对于工业应用来说十分重要, 而且高温条件下, 酶的反应速度更快, 能缩短反应周期, 节约成本, 也有利于避免反应过程中被其他微生物污染。

[0003] 随着蛋白质工程技术和分子生物学的发展, 运用定向进化和理性设计的手段对酶分子进行人工进化和改造已成为当前酶工程领域研究的热点。到目前为止, 已有许多学者运用这项技术成功的改造了各种各样的酶, 取得了令人瞩目的进展 (Zhao, 2007, *Biotechnology and Bioengineering* 98(2), 271-275)。其中易错 PCR (error-prone PCR)、DNA 改组 (DNA shuffling)、半理性设计等已成为酶的分子改造中常用的手段, 大大加速了蛋白质的进化过程 (Lehmann and Wyss, 2001 *Current Opinion in Biotechnology* 12(4), 371-375)。

发明内容

[0004] 本发明的目的在于采用定向进化技术同时结合基于序列比对的半理性设计方法对来源于 *Geobacillus stearothermophilus* 的 1, 4- β -D- 木聚糖酶 XT6 进行分子改造, 获得热稳定性提高的木聚糖酶突变体。

[0005] 为达到上述目的, 本发明使用多轮易错 PCR 方法、DNA 改组技术对 1, 4- β -D- 木聚糖酶 XT6 基因进行突变, 再通过高通量筛选方法将正突变检出, 同时使用半理性设计方法确定部分潜在热稳定相关位点, 再通过定点突变方法得到热稳定相关突变体。

[0006] 本发明的具体实施方法为: 来源于 *G. stearothermophilus* (Genbank 登录号为: Z29080) 的木聚糖酶 XT6 由 379 个氨基酸组成 (Lapidot, Mechaly et al., 1996 *Journal of Biotechnology* 51(3), 259-264) (见 SEQ IDNO.1)。为迅速得到 XT6 全基因序列并提高其在大肠杆菌中的表达量, 以利于对 XT6 酶分子的改造, 根据已报道的 XT6 基因序列信息 (Gat, Lapidot et al., 1994 *Applied and Environmental Microbiology* 60(6), 1889-1896), 采用基于 PCR 的全基因合成方法合成了木聚糖酶 XT6 全基因序列。同时根据大肠杆菌密码子偏好性对其 DNA 序列进行优化 (在线软件 DNABworks, <http://>

helixweb.nih.gov/dnaworks/,优化合成的木聚糖酶 XT6 基因序列为 SEQ ID NO. 2),功能正常的酶蛋白在大肠杆菌 BL21-DE3 中过量表达 Zhang, Peiet al., 2009 Chinese Journal of Applied and Environmental Biology 15(2), 271-275)。采用易错 PCR 方法、DNA 改组技术对其进行随机突变,以 pET 28a(+) 载体构建高效突变库,再将含有突变基因的质粒转入表达宿主 E. coli BL21-DE3 中,挑选单克隆于 96 孔板中表达蛋白。离心重悬后,取部分菌液加入 1% 浓度木聚糖底物反应适当时间后,以 DNS 法终止反应,测定酶活性。同时,另取部分菌液热处理一定时间,测定残余活性。残余活性高于对照的菌株转接到新的 96 孔培养板中,进行重复筛选。将筛选得到的残余活性高于对照的菌株,送上海英骏生物技术有限公司测序,获得突变体 DNA 序列信息。详细方案见实施例 2。

[0007] 同时采用基于序列比对的半理性设计方法获得热稳定突变体 ((Lehmann, Loch et al., 2002 Proteinengineering 15(5), 403-411)。具体做法为,首先将 XT6 氨基酸序列与其他不同来源的 17 条木聚糖酶氨基酸序列进行比对,初步确定有可能影响酶稳定性的位点,通过定点突变得得到各个位点的单点突变体,测定其热失活半衰期,并与野生型比较,得到酶热稳定性的突变体 F360L。详细方案见实施例 6。

[0008] 经上述对构建的突变文库筛选以及半理性设计方法,获得 20 个热稳定性提高的突变体,分别为 10E11、05D03、09D05、08A07、05F03、04D08、04F06、04H09、08G01、06B01、04D03、03B11、04H12、02E04、06H07、F360L、FAM、FAMG、FTAMG 和 FC06T,序列信息见 SEQ ID NO. 3-NO. 22,其特征如下:

[0009] 10E11:该酶的第 53 位的苯丙氨酸突变为酪氨酸(DNA 序列由 TTT 变为 TAT)。

[0010] 05D03:该酶的第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GAT)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)。

[0011] 09D05:该酶的第 121 位的苏氨酸突变为异亮氨酸(ACC 变为 ATC)、第 364 位的甘氨酸突变为天冬氨酸(GGC 变为 GAC)。

[0012] 08A07:该酶的第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)。

[0013] 05F03:该酶的第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GTA)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)、第 364 位的甘氨酸突变为天冬氨酸(GGC 变为 GAC)。

[0014] 04D08:该酶的第 53 位的苯丙氨酸突变为酪氨酸(TTT 变为 TAT)、第 73 位的赖氨酸突变为异亮氨酸(AAA 变为 ATA)、第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GTA)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)。

[0015] 04F06:该酶的第 53 位的苯丙氨酸突变为酪氨酸(TTT 变为 TAT)、第 364 位的甘氨酸突变为天冬氨酸(GGC 变为 GAC)、第 380 位的赖氨酸突变为苏氨酸(AAA 变为 ACA)。

[0016] 04H09:该酶的第 53 位的苯丙氨酸突变为酪氨酸(TTT 变为 TAT)、第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GTA)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)、第 380 位的赖氨酸突变为异亮氨酸(AAA 变为 ATA)。

[0017] 08G01:该酶的第 53 位的苯丙氨酸突变为酪氨酸(TTT 变为 TAT)、第 113 位的谷氨酸突变为天冬氨酸(GAA 变为 GAT)、第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GTA)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)。

[0018] 06B01:该酶的第 213 位的苏氨酸突变为异亮氨酸(ACT 变为 ATT)、第 257 位的丙氨酸突变为亮氨酸(GCA 变为 TTA)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)、第 364

位的甘氨酸突变为天冬氨酸(GGC 变为 GAC)。

[0019] 04D03 :该酶的第 26 位的赖氨酸突变为异亮氨酸(AAA 变为 ATA)、第 53 位的苯丙氨酸突变为酪氨酸(TTT 变为 TAT)、第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GTA)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)、第 364 位的甘氨酸突变为天冬氨酸(GGC 变为 GAC)。

[0020] 03B11 :该酶的第 138 位的谷氨酸突变为天冬氨酸(GAA 变为 GAC)、第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GTA)、第 271 位的蛋氨酸突变为异亮氨酸, (ATG 变为 ATA)、第 364 位的甘氨酸突变为天冬氨酸(GGC 变为 GAC)。

[0021] 04H12 :该酶的第 53 位的苯丙氨酸突变为酪氨酸(TTT 变为 TAT)、第 116 位的脯氨酸突变为苏氨酸(CCG 变为 ACG)、第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GTA)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)。

[0022] 02E04 :该酶的第 17 位的天冬酰胺突变为赖氨酸(AAC 变为 AAA)、第 53 位的苯丙氨酸突变为酪氨酸(TTT 变为 TAT)、第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GTA)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)。

[0023] 06H07 :该酶的第 53 位的苯丙氨酸突变为酪氨酸(TTT 变为 TAT)、第 73 位的赖氨酸突变为异亮氨酸(AAA 变为 ATA)、第 121 位的苏氨酸突变为异亮氨酸(ACC 变为 ATC)、第 219 位的缬氨酸突变为丙氨酸(GAT 变为 GCT)、第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GTA)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)、第 364 位的甘氨酸突变为天冬氨酸(GGC 变为 GAC)、第 380 位的赖氨酸突变为天冬酰胺(AAA 变为 AAC)。

[0024] F360L :该酶的第 361 位的苯丙氨酸突变为亮氨酸(TTT 变为 TTA)。

[0025] FAM :该酶的第 53 位的苯丙氨酸突变为酪氨酸(TTT 变为 TAT)、第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GTA)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)。

[0026] FAMG :该酶的第 53 位的苯丙氨酸突变为酪氨酸(TTT 变为 TAT)、第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GTA)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)、第 364 位的甘氨酸突变为天冬氨酸(GGC 变为 GAC)。

[0027] FTAMG :该酶的第 53 位的苯丙氨酸突变为酪氨酸(TTT 变为 TAT)、第 121 位的苏氨酸突变为异亮氨酸(ACC 变为 ATC)、第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GTA)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)、第 364 位的甘氨酸突变为天冬氨酸(GGC 变为 GAC)。

[0028] FC06T :该酶的第 17 位的天冬酰胺突变为赖氨酸(AAC 变为 AAA)、第 26 位的赖氨酸突变为异亮氨酸(AAA 变为 ATA)、第 53 位的苯丙氨酸突变为酪氨酸(TTT 变为 TAT)、第 73 位的赖氨酸突变为异亮氨酸(AAA 变为 ATA)、第 113 位的谷氨酸突变为天冬氨酸(GAA 变为 GAT)、第 121 位的苏氨酸突变为异亮氨酸(ACC 变为 ATC)、第 138 位的谷氨酸突变为天冬氨酸(GAA 变为 GAC)、第 219 位的缬氨酸突变为丙氨酸(GAT 变为 GCT)、第 257 位的丙氨酸突变为缬氨酸(GCA 变为 GTA)、第 271 位的蛋氨酸突变为异亮氨酸(ATG 变为 ATA)、第 361 位的苯丙氨酸突变为亮氨酸(TTT 变为 TTA)、第 364 位的甘氨酸突变为天冬氨酸(GGC 变为 GAC)、第 380 位的赖氨酸突变为天冬酰胺(AAA 变为 AAC)。

[0029] 将上述突变体的基因连入载体 pET 28a(+), 并在宿主细胞大肠杆菌 Escherichiacoli BL21-DE3 中表达, 获得突变体酶蛋白。

[0030] 相比野生型 1, 4- β -D-木聚糖酶 XT6, 本发明的 20 个突变体热稳定性明显提高, 在 75° C 的热失活半衰期 ($t_{1/2}$) 分别提高了 2.4、2.5、2.1、3.1、5.1、6.7、8、8.8、10.4、9.2、19.9、13.7、8.5、10.4、23、2.3、7.3、15、19 和 52 倍, 显示出在造纸制浆, 生物能源等工业上潜在的应用价值。

附图说明

[0031] 图 1 为包含所有已发现的热稳定相关突变位点的 7 个突变体不同温度下残余酶活的测定。

[0032] 图 2 为包含所有已发现的热稳定相关突变位点的 7 个突变体在不同温度下酶的反应速率测定。

[0033] SEQ ID NO.1 为来源于 *Geobacillus stearothermophilus* (Genebank 登录号为: Z29080) 的木聚糖酶 XT6 氨基酸序列, SEQ ID NO.2 为本发明优化的木聚糖酶基因 XT6 核苷酸序列。SEQ ID NO.3 为突变体 10E11 的氨基酸序列。SEQ ID NO.4 为突变体 05D03 的氨基酸序列。

[0034] SEQ ID NO.5 为突变体 09D05 的氨基酸序列。SEQ ID NO.6 为突变体 08A07 的氨基酸序列。SEQ ID NO.7 为 05F03 的氨基酸序列。SEQ ID NO.8 为 04D08 的氨基酸序列。SEQ ID NO.9 为 04F06 的氨基酸序列。SEQ ID NO.10 为 04H09 的氨基酸序列。SEQ ID NO.11 为 08G01 的氨基酸序列。SEQ ID NO.12 为 06B01 的氨基酸序列。SEQ ID NO.13 为 04D03 的氨基酸序列。SEQ ID NO.14 为 03B11 的氨基酸序列。SEQ ID NO.15 为 04H12 的氨基酸序列。SEQ ID NO.16 为 02E04 的氨基酸序列。SEQ ID NO.17 为 06H07 的氨基酸序列。SEQ ID NO.18 为 F360L 的氨基酸序列。SEQ ID NO.19 为 FAM 的氨基酸序列。SEQ ID NO.20 为 FAMG 的氨基酸序列。SEQ ID NO.21 为 FTAMG 的氨基酸序列。SEQ ID NO.22 为 FC06T 的氨基酸序列。

具体实施方式

[0035] 以下结合实施实例对本发明做进一步说明, 需要指出的是, 本实施例仅用于解释本发明, 而非对本发明范围的限制。

[0036] 实施例 1 易错 PCR (error-prone PCR) 方法构建木聚糖酶 XT6 突变文库

[0037] 采用 GeneMorph® II Random Mutagenesis kit, 以优化后的 XT6 (见 SEQ ID NO.2) 为模板扩增 1, 4- β -D-木聚糖酶 XT6 基因, 随机引入突变。

[0038] 所用引物为: 5' -TAGGAGGTCATATGAAAAATGCGGACAGCTATGCG-3' ,

[0039] 5' -ATACGCGGATCCCTATTTGTGATCAATGATCGCCCAATACGCCGCTT-3'

[0040] 反应条件为: 94° C 预变性 10min, 94° C 变性 30s, 60° C 退火 60s 和 72° C 延伸 2min, 共 25 个循环, 0.8% 琼脂糖电泳, 试剂盒回收目的基因片段。

[0041] 按照 NEB 公司 2007-2008 产品目录说明书描述的方法, 用 Nde I 和 BamH III 双酶切消化后, 与经过相同酶切的 pET 28a(+) 载体 (卡那霉素抗性基因) 进行连接反应, 反应条件为: 载体和片段按摩尔比 1:3 的比例混合, 加入 400 个单位的 T4 连接酶, 16° C 过夜。电击法转入大肠杆菌 DH10B, 得到超过 105 个克隆的突变体库。

[0042] 实施例 2 木聚糖酶 XT6 突变体库的筛选

[0043] 将实施例 1 中突变库克隆收集后提取质粒,转入大肠杆菌表达菌株 BL21-DE3,涂布含有卡那霉素的 LB 平板,培养 12h。挑取单克隆于 96 孔板,每孔含有 150 μ L TB 培养基(含有 50 μ g/mL 卡那霉素,1mM IPTG),37 $^{\circ}$ C,245rpm,震荡培养 36h。96 孔板复制器复制各单克隆于 LB 固体培养基平板,37 $^{\circ}$ C 培养 12h 后,4 $^{\circ}$ C 冰箱保存。用排枪轻轻吸出 96 孔板各孔中的细胞培养物,按相应位置分配于 A 板和 B 板的 96 孔板中,每块 96 孔板各孔中的培养物为 70 μ L。4000rpm,4 $^{\circ}$ C,离心 10min,弃去上清,各孔中菌体细胞用 30 μ L,50mM, pH 7.6 的磷酸钠缓冲液重悬。A 板直接加入 50 μ L,50mM, pH 7.6 的磷酸钠缓冲液配制的 1% 木聚糖底物,37 $^{\circ}$ C,245rpm,反应 1.5h,加入 120 μ L DNS 溶液,充分混匀后置于烘箱,显色,筛选酶活性高于野生型对照的突变体;B 板用保鲜膜封好,置于 85 $^{\circ}$ C 烘箱,2h 处理后迅速放置在 -20 $^{\circ}$ C,温度快速降至室温。加入 50 μ L 用 50mM, pH 7.6 的磷酸钠缓冲液配制的 1% xylan 底物,37 $^{\circ}$ C,245rpm,反应 3h,加入 120 μ L DNS 溶液,加热显色。测定突变体残余活性,取残余活性比野生型 XT6 高的菌株到新的 96 孔培养板中,进行重复筛选。筛选到 4 个突变体,分别为 10E11、05D03、09D05、08A07,残余活性是野生型对照的 2-3 倍,分别挑取单克隆送上海英骏生物技术公司测序。

[0044] 实施例 3 突变体和野生型热酶蛋白粗体物的制备和热稳定性的测定

[0045] 3.1 粗酶液制备和酶活测定方法

[0046] 挑取筛选到的突变体单克隆于 20mL TB (50mg/mL 卡那霉素、1.0mM IPTG)、37 $^{\circ}$ C 培养并诱导 36h 后,6000rpm,离心 10min 收集菌体。弃去上清,12mL 磷酸钠缓冲液(50mM, pH7.6)重悬菌体,超声波破碎细胞,提取酶蛋白粗提液。

[0047] 酶活测定:10mL 离心管中,反应体系为:0.2mL 稀释的粗酶液(用磷酸盐缓冲液稀释 50 倍),1.8mL 底物溶液(提前在 50 $^{\circ}$ C 温浴 30min),50 $^{\circ}$ C 水浴,5min,再加入 3mL DNS 溶液终止反应,混匀后,沸水浴中煮沸 5min,立即用冷水冷却至室温,540nm 处测定吸光值。每个样品三个重复 (Bailey, Biely et al., 1992 Journal of biotechnology 23(3), 257-270)。酶活力单位定义:在 pH 7.6, 50 $^{\circ}$ C 条件下,每分钟产生 1mmol 还原糖或者其相等物所需要的酶量为一个活力单位(U)。

[0048] 3.2 XT6 酶突变体热稳定性的测定

[0049] 将蛋白浓度 0.1mg/mL 的粗酶液 40mL 置于容量 250mL 的 PCR 管,每个样品 3 个重复,用 PCR 仪在 75 $^{\circ}$ C 处理不同的时间,然后将样品管放置于冰上冷却,4 $^{\circ}$ C,12000rpm,离心 25min,取适量处理后的酶液测定残余活性,测定方法见 3.1。以未经过高温处理的酶液为参比,得到残余酶活百分比。以处理时间为 X 轴,残余酶活百分比的自然对数为 Y 轴,用 origin75 软件做散点图,添加趋势线,得到酶热处理时间与残余活性的线性方程,以残余 50% 的自然对数为 3.912023 来计算得到相应的时间,此时间即为突变体的热失活半衰期 $t_{1/2}$ 。各突变体在 pH 7.6, 75 $^{\circ}$ C 条件下的热失活半衰期见表 1。突变体 10E11, 05D03, 09D05 和 08A07 在 75 $^{\circ}$ C 的热失活半衰期分别为野生型的 2.4、2.5、2.1 和 3.1 倍。

[0050] 将蛋白浓度 0.1mg/mL 的粗酶液 300mL 置于 1.5mL 离心管中,每个样品 3 个重复,在 pH 7.6, 不同温度下处理 20min,迅速放置冰上冷却,4 $^{\circ}$ C,12000rpm,离心 25min,取适量酶液测定残余活性,参照实施案例 3.1。在以未经过高温处理的酶液为参比,得到残余酶活百分比,结果见说明书附图 2,与野生型比较,突变体 10E11, 05D03, 09D05 和 08A07 的热稳定性有显著提高,野生型木聚糖酶在 72 $^{\circ}$ C 处理 20min 后,只剩下 10% 的活性,而 4 个突变体

在相同条件下,仍能保持 50% 的残余活性。

[0051] 3.3 温度对酶突变体反应速率的影响

[0052] 在 pH 7.5 条件下,测定木聚糖酶野生型和突变型在不同温度下的最大反应速率,酶的浓度为 0.1mg/mL,反应温度分别为 60° C,65° C,70° C,75° C,80° C,85° C,90° C,95° C。测定方法同 3.1。以最大反应速率时的活性为 100%,其他温度下测定的酶活与它的比值百分比为纵坐标,反应温度为横坐标,绘制酶活随反应温度变化的曲线。结果见说明书附图 2。突变体 10E11,05D03,09D05 和 08A07 在 82° C 具有最大反应速率,比野生型提高 5° C。

[0053] 实施例 4 第二轮易错 PCR 突变文库的构建和筛选

[0054] 4.1 突变文库的构建

[0055] 除以第一轮易错 PCR 筛选到的三个突变体 10E11、05D03、09D05 提取质粒作第二轮易错 PCR 的模板外,随机突变体库的构建过程中,引物的选择,PCR 反应条件,文库的构建同实施例 1。结果得到超过 105 个克隆的随机突变体库。

[0056] 4.2 突变文库的筛选

[0057] 将步骤 4.1 中构建得到的突变体转入大肠杆菌表达菌株 BL21-DE3,筛选活性 / 热稳定正突变时以 10E11、05D03、09D05 为参照,热处理时间为 150min,其余操作同实施例 2,取活性 / 残余活性比突变型 10E11、05D03、09D05、高的菌株到新的 96 孔培养板中,进行重复筛选。筛选到 10 个热稳定性提高的酶突变体,分别为 05F03、04D08、04F06、04H09、08G01、06B01、04D03、03B11、04H12、02E04。挑取热稳定正突变体送于上海英骏生物技术有限公司测序。

[0058] 4.3 突变体热稳定性的测定

[0059] 测定方法同实施例 3.2,结果(表 1)表明,突变体 05F03、04D08、04F06、04H09、08G01、06B01、04D03、03B11、04H12、02E04 在 75° C 的热失活半衰期是野生型的 5.1、6.7、8、8.8、10.4、9.2、19.9、13.7、8.5 和 10.4 倍。

[0060] 实施例 5XT6 突变体基因 DNA 改组突变库的构建和筛选

[0061] 5.1DNA 改组突变库的构建

[0062] 以筛选到的木聚糖酶 XT6 突变体 10E11、05D03、09D05、08A07、05F03、04D08、04F06、04H09、08G01、06B01、04D03、03B11、04H12、02E04 为模板,一对引物:

[0063] 5' -GTGAGCGGATAACAATTCCC-3' ,

[0064] 5' -CCTCAAGACCCGTTTAGAGG-3'

[0065] 分别扩增各突变体基因,反应条件为:94° C 预变性 10min,94° C 变性 30s,60° C 退火 30s 和 72° C 延伸 2min,共 25 个循环,0.8% 琼脂糖电泳,试剂盒回收纯化目的基因。按照 Stemmer 的方法,DNase I 将获得的 DNA 目的基因片段处理适当时间,回收 50~150bp 的小片段 (Stemmer,1994 Proceedings of theNational Academy of Science of the United States of America 91(22),10747-10751)。经过无引物 PCR 和有引物 PCR,所用引物为:

[0066] 5' -TAGGAGGTCATATGAAAAATGCGGACAGCTATGCG-3' ,

[0067] 5' -ATACGCGGATCCCTATTTGTGATCAATGATCGCCCAATACGCCGGCTT-3'

[0068] 得到重组后的全长目的基因。DNA 改组突变文库的构建方法同实施例 1,获得超过 105 个克隆的突变体库。

[0069] 5.2 DNA 改组突变库的筛选

[0070] 筛选正突变以第二轮易错 PCR 中获得的热稳定性最高的突变体 04D03 为参照, 热处理时间为 240min, 其余操作同实施例 2, 取残余活性比突变型 04D03 高的菌株到新的 96 孔培养板中, 进行重复筛选。筛选到突变体 06H07。送于上海英骏生物技术有限公司测序。

[0071] 5.3 突变体热稳定性的测定

[0072] 测定方法同实施例 3.2, 结果(表 1)表明, 突变体 06H07 热稳定性和野生型相比有很大的提高, 在 75° C 条件下的热失活半衰期是野生型的 23 倍。

[0073] 实施例 6 基于序列比对的半理性设计

[0074] 将木聚糖酶 XT6 氨基酸序列与蛋白质文库(SwissPort)中已知的 F10 家族 17 个亲缘关系比较近的木聚糖酶氨基酸序列进行比对(表 2)。在这 17 个不同来源的木聚糖酶氨基酸序列中, 9 个木聚糖酶来源于嗜热菌, 8 个来自中温细菌。他们的氨基酸序列和木聚糖酶 XT6 相比, 氨基酸相似度在 40%-75% 之间。序列比对所用软件为 Clustal X。

[0075] 比对结果发现, 木聚糖酶 XT6 氨基酸序列中 Phe95, Ile152, Tyr200, Asn262, Phe361 和 Ile376 位的氨基酸在其同源木聚糖酶氨基酸序列相应位置占多数的氨基酸分别为 Gly, Val, Phe, Ile, Leu 和 Val。定点突变方法改变这些位点, 所用引物:

[0076] XYL94-F :5' -GGCATGGATATTCGTGGTCACACCCTGGTGTGG-3'

[0077] XYL94-R :5' -CCACACCAGGGTGTGACCACGAATATCCATGCC-3'

[0078] XYL151-F :5' -GAACGTTATAAGGATGATGTAAAGTACTGGGATGTAG-3'

[0079] XYL151-R :5' -CTACATCCCAGTACTTTACATCATCCTTATAACGTTC-3'

[0080] XYL199-F :5' -GGCGATAACATTAAGTTATTCATGAATGACTACAATACCG-3'

[0081] XYL199-R :5' -CGGTATTGTAGTCATTCATGAATAACTTAATGTTATCGCC-3'

[0082] XYL261-F :5' -GCACTGGGCCTGGATATCCAAATTACCGAACTGG-3'

[0083] XYL261-R :5' -CCAGTTCGGTAATTTGGATATCCAGGCCAGTGC-3'

[0084] 360-F :5' -GCAAAGATGCGCCGCTTGTGTTTGGCCCGG-3'

[0085] 360-R :5' -CCGGGCCAAACACAAGCGGCGCATCTTTGC-3'

[0086] XYL375-F :5' -CCGGCGTATTGGGCGGTCATTGATCACAAATAG-3'

[0087] XYL375-R :5' -CTATTTGTGATCAATGACCGCCCAATACGCCGG-3'

[0088] PCR 条件为: 10×Buffer 5mL, 引物(10mM)各 6mL, dNTP (2.5mM) 6mL, pfu (2.5U/mL) 1mL, 质粒 10ng, 超纯水补足 50mL, 条件: 95° C 预变性 30s, 95° C 变性 30s, 55° C 退火 1min, 68° C 延伸 7min, 共 12 个循环。PCR 产物用 1mL DpnI 处理, 37° C 处理 1h。PCR 产物 10mL 化学法转入 E. coli DH5a。送于上海英骏生物技术有限公司测序。测序正确后, 提取质粒转入表达菌株 E. coli BL21-DE3。提取突变体酶蛋白, 测定其在 75° C 下的热稳定性。测定方法同实施例 3.2。结果得到热稳定性提高的突变体 F360L, 在 75° C 的热失活半衰期为 10.5min, 是野生型的 2.3 倍(表 3)。

[0089] 实施例 7 突变位点组合构建 XT6 新突变体

[0090] 7.1 突变体 FAM 的构建

[0091] 定点突变方法将突变体 05D03 的 53 位的苯丙氨酸突变为酪氨酸, 构建突变体 FAM, 所用的引物如下:

[0092] 52-F :5' -GATGCTGAAGCGTCATTATAACTCAATTGTGGCGG-3' ,

[0093] 52-R:5' -CCGCCACAATTGAGTTATAATGACGCTTCAGCATC-3'

[0094] PCR 条件以及操作同实施例 6, 得到新突变体 FAM。

[0095] 7.2 突变体 FAMG 的构建

[0096] 定点突变法将突变体 FAM 的 364 位的甘氨酸突变为天冬氨酸, 构建突变体 FAMG。所用的引物如下:

[0097] 363-F:5' -GCGCCGTTTGTGTTTGACCCGGATTACAAAGTGAAGC-3' ,

[0098] 363-R:5' -GCTTCACTTTGTAATCCGGGTCAAACACAAACGGCGC-3'

[0099] PCR 条件以及后续操作同实施例 6, 得到突变体 FAMG。

[0100] 7.3 突变体 FTAMG 的构建

[0101] 定点突变法将突变体 FAMG 的 121 位的苏氨酸突变为异亮氨酸, 构建突变体 FTAMG, 所用的引物如下:

[0102] 120-F:5' -CCGATGGTGAACGAGATCGATCCGGTGAAACGTG-3' ,

[0103] 120-R:5' -CACGTTTCACCGGATCGATCTCGTTCACCATCGG-3'

[0104] PCR 条件以及后续操作同实施例 6, 得到突变体 FTAMG。

[0105] 7.4 突变体 FC06T 的构建

[0106] 定点突变法将突变体 06H07 的 17 位的天冬酰胺突变为赖氨酸, 26 位的赖氨酸突变为异亮氨酸, 113 位的脯氨酸突变为苏氨酸, 138 位的谷氨酸突变为天冬氨酸, 361 位的苯丙氨酸突变为亮氨酸, 构建突变体 FC06T, 所用的引物如下:

[0107] 16-F:5' -GCATATTAGCGCCCTGAAAGCGCCACAGCTGG-3'

[0108] 16-R:5' -CCAGCTGTGGCGCTTTCAGGGCGCTAATATGC-3'

[0109] 25-F:5' -CAGCTGGACCAACGGTACATAAATGAATTTACTATTGGTGCG-3'

[0110] 25-R:5' -CGCACCAATAGTAAATTCATTTATGTACCGTTGGTCCAGCTG-3'

[0111] 112-F:5' -GGTTCTTCCTTGACAAAGATGGAAAACCGATGGTG-3'

[0112] 112-R:5' -CACCATCGGTTTTCCATCTTTGTCAAGGAAGAACC-3'

[0113] 137-F:5' -CTGCTGAAACGCTTGGACACCCACATCAAAACC-3'

[0114] 137-R:5' -GGTTTTGATGTGGGTGTCCAAGCGTTTCAGCAG-3'

[0115] 360-F:5' -GCAAAGATGCGCCGCTTGTGTTTGGCCCGG-3'

[0116] 360-R:5' -CCGGGCCAAACACAAGCGGCGCATCTTTGC-3'

[0117] PCR 条件以及后续操作同实施例 6, 得到突变体 FC06T。

[0118] 7.5XT6 突变体热稳定性测定

[0119] 木聚糖酶突变体 FAM, FAMG, FTAMG, FC06T 热稳定性测定方法同 3.2, 测定结果表明: FAM, FAMG, FTAMG, FC06T 在 75° C 条件下的热失活半衰期分别是野生型的 7.3、14.8、19.4 和 52 倍(表 1); FTAMG, FC06T 经 20min 热处理后测定残余活性, 78° C 时, 野生型残余活性低于 1%, 而突变体 FTAMG 仍能保持 20% 活性, FC06T 仍能保持 50% 的活性, 当温度达到 80° C 时, FTAMG 的残余活性低于 5%, 而突变体 FC06T 的残余活性为 30%。温度对酶突变体反应速率的影响测定同 3.3, 突变体 FTAMG, FC06T 在 87° C 具有最大反应速率, 比野生型提高 10° C。结果见说明书附图 2。

[0120] 表 1 突变体在 75° C 热失活半衰期

[0121]

来源	编号	改变的氨基酸	t _{1/2} (min)	提高倍数
第一轮易错 PCR	WT	无	3.5	1
	10E11	F53Y	8.5	2.4
	05D03	A257V/M271I	8.9	2.5
	09D05	T121I/G364D	7.4	2.1
	08A07	M271I	10.7	3.1
第二轮易错 PCR	WT	无	4.5	1
	05F03	A257V/ M271I/ G364D	23	5.1
	04D08	F53Y/K73I/A257V/ M271I	30	6.7
	04F06	F53Y/ G364D/K380T	35.8	8.0
	04H09	F53Y/A257V/ M271I/K380I	30.8	8.8
	08G01	F53Y/E113D/A257V/ M271I	46.6	10.4
	06B01	T213I/A257L/M271I/ G364D	41.3	9.2
	04D03	K26I/F53Y/A257V/ M271I/ G364D	85.3	19.9
	03B11	E138D/A257V/ M271I/ G364D	61.8	13.7
	04H12	F53Y/P116T/A257V/ M271I	38.9	8.5
	02E04	N17K/F53Y/A257V/ M271I	46.7	10.4
DNA 改组	WT	无	4.2	1
	06H07	F53Y/K73I/T121I/A257V/M271I/ G364D/ V219A/K380N	92.7	23
半理性设计	WT	无	4.5	1
	F360L	F361L	10.5	2.3
突变位点组合	FAM	F53Y/A257V/ M271I	32.5	7.3
	FAMG	F53Y/A257V/ M271I/ G364D	66.7	14.8
	FTAMG	F53Y/T121I/A257V/ M271I/ G364D	87.5	19.4
	WT	无	3.5	1
	FC06T	N17K/K26I/F53Y/K73I/E113D/T121I/ /E138D/V219A/A257V/M271I/F361L / G364D/K380N	182	52

[0122] 表 2 不同来源的 F10 家族木聚糖酶特征

序列登 录号	木聚糖酶氨基酸序列来源	序列相似 度	氨基酸	序列一致 性
P26514	<i>Streptomyces lividans</i>	47 %	477	33 %
P10474	<i>Caldicellulosiruptor saccharolyticus</i>	56 %	1039	39 %
P48789	<i>Prevotella ruminicola</i>	55 %	369	39 %
P07528	<i>Bacillus halodurans</i>	75 %	396	61 %
Q60042	<i>Thermotoga neapolitana</i>	56 %	1055	39 %
Q60037	<i>Thermotoga maritima</i>	57 %	1059	40 %
[0123] P40944	<i>Caldicellulosiruptor sp. Rt8B.4</i>	56 %	684	39 %
P10478	<i>Clostridium thermocellum ATCC 27405</i>	48 %	837	33 %
P23557	<i>Caldicellulosiruptor saccharolyticus</i>	50 %	312	34 %
P26223	<i>Butyrivibrio fibrisolvens</i>	50 %	635	34 %
P23556	<i>Caldicellulosiruptor saccharolyticus</i>	61 %	342	43 %
P45703	<i>Geobacillus stearothermophilus</i>	56 %	330	37 %
P36917	<i>Thermoanaerobacterium saccharolyticum</i>	57 %	1157	41 %
P38535	<i>Clostridium thermocellum</i>	56 %	1087	41 %
P14768	<i>Pseudomonas fluorescens</i>	44 %	611	30 %
P07529	<i>Cryptococcus albidus</i>	58 %	332	40 %
P23030	<i>Pseudomonas fluorescens</i>	41 %	599	28 %

[0124] 表 3 单点突变体热失活半衰期

XT6 酶突变体	酶蛋白比活性 (U/mg)	$t_{1/2}$ (min)
WT	420	<u>4.5</u>
[0125] F95G	384	3.2
Y200F	407	5.4
N262I	448	3.3
F361L	498	<u>10.5</u>
I376V	528	5.3

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[0001]

SEQUENCE LISTING

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<212> PRT

<213> Geobacillus stearothermophilus

<400> 3

Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr
 1 5 10 15 20 25
 Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys
 30 35 40 45 50
 Arg His Tyr Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn
 55 60 65 70 75
 Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp
 80 85 90 95 100
 His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys
 105 110 115 120 125
 Arg Glu Gln Asn Lys Gln Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp
 130 135 140 145 150
 Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln
 155 160 165 170 175
 Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr

[0003]

180	185	190	195	200
Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu				
205	210	215	220	225
Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys				
230	235	240	245	250
Thr Ile Asn Met Phe Ala Ala Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Met Tyr Gly Trp Pro				
255	260	265	270	275
Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu				
280	285	290	295	300
Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp				
305	310	315	320	325
Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys				
330	335	340	345	350
Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala				
355	360	365	370	375
Ile Ile Asp His Lys				
380				
<210>	4			
<211>	380			
<212>	PRT			
<213>	Geobacillus stearothermophilus			
<400>	4			
Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr				
1	5	10	15	20
Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys				
30	35	40	45	50
Arg His Phe Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn				
55	60	65	70	75
Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp				
80	85	90	95	100
His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys				
105	110	115	120	125
Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp				
130	135	140	145	150
Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln				
155	160	165	170	175
Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr				
180	185	190	195	200
Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu				
205	210	215	220	225
Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys				
230	235	240	245	250
Thr Ile Asn Met Phe Ala Val Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro				
255	260	265	270	275

[0004]

Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu
 280 285 290 295 300
 Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp
 305 310 315 320 325
 Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys
 330 335 340 345 350
 Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala
 355 360 365 370 375
 Ile Ile Asp His Lys
 380

<210> 5

<211> 380

<212> PRT

<213> *Geobacillus stearothermophilus*

<400> 5

Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr
 1 5 10 15 20 25
 Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys
 30 35 40 45 50
 Arg His Phe Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn
 55 60 65 70 75
 Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp
 80 85 90 95 100
 His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Ile Asp Pro Val Lys
 105 110 115 120 125
 Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp
 130 135 140 145 150
 Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln
 155 160 165 170 175
 Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr
 180 185 190 195 200
 Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu
 205 210 215 220 225
 Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys
 230 235 240 245 250
 Thr Ile Asn Met Phe Ala Ala Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Met Tyr Gly Trp Pro
 255 260 265 270 275
 Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu
 280 285 290 295 300
 Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp
 305 310 315 320 325
 Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys
 330 335 340 345 350
 Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Asp Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala

[0005]

355	360	365	370	375
Ile Ile Asp His Lys				
380				
<210> 6				
<211> 380				
<212> PRT				
<213> Geobacillus stearothermophilus				
<400> 6				
Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr				
1 5 10 15 20 25				
Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys				
30 35 40 45 50				
Arg His Phe Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn				
55 60 65 70 75				
Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp				
80 85 90 95 100				
His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys				
105 110 115 120 125				
Arg Glu Gln Asn Lys Gln Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp				
130 135 140 145 150				
Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln				
155 160 165 170 175				
Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr				
180 185 190 195 200				
Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu				
205 210 215 220 225				
Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys				
230 235 240 245 250				
Thr Ile Asn Met Phe Ala Ala Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro				
255 260 265 270 275				
Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu				
280 285 290 295 300				
Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp				
305 310 315 320 325				
Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys				
330 335 340 345 350				
Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala				
355 360 365 370 375				
Ile Ile Asp His Lys				
380				
<210> 7				
<211> 380				
<212> PRT				
<213> Geobacillus stearothermophilus				

[0006]

<400> 7

Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr
 1 5 10 15 20 25
 Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys
 30 35 40 45 50
 Arg His Phe Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn
 55 60 65 70 75
 Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp
 80 85 90 95 100
 His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys
 105 110 115 120 125
 Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp
 130 135 140 145 150
 Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln
 155 160 165 170 175
 Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr
 180 185 190 195 200
 Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu
 205 210 215 220 225
 Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys
 230 235 240 245 250
 Thr Ile Asn Met Phe Ala Val Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro
 255 260 265 270 275
 Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu
 280 285 290 295 300
 Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp
 305 310 315 320 325
 Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys
 330 335 340 345 350
 Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Asp Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala
 355 360 365 370 375
 Ile Ile Asp His Lys
 380

<210> 8

<211> 380

<212> PRT

<213> *Geobacillus stearothermophilus*

<400> 8

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

[0007]

55	60	65	70	75
Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp				
80	85	90	95	100
His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys				
105	110	115	120	125
Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp				
130	135	140	145	150
Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln				
155	160	165	170	175
Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr				
180	185	190	195	200
Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu				
205	210	215	220	225
Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys				
230	235	240	245	250
Thr Ile Asn Met Phe Ala Val Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro				
255	260	265	270	275
Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu				
280	285	290	295	300
Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp				
305	310	315	320	325
Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys				
330	335	340	345	350
Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala				
355	360	365	370	375

Ile Ile Asp His Lys

380

<210> 9

<211> 380

<212> PRT

<213> Geobacillus stearothermophilus

<400> 9

Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr

1 5 10 15 20 25

Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys

30 35 40 45 50

Arg His Tyr Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn

55 60 65 70 75

Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp

80 85 90 95 100

His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys

105 110 115 120 125

Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp

130 135 140 145 150

[0008]

Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln
 155 160 165 170 175
 Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr
 180 185 190 195 200
 Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu
 205 210 215 220 225
 Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys
 230 235 240 245 250
 Thr Ile Asn Met Phe Ala Ala Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Met Tyr Gly Trp Pro
 255 260 265 270 275
 260 265 270
 Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu
 280 285 290 295 300
 Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp
 305 310 315 320 325
 Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys
 330 335 340 345 350
 Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Asp Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala
 355 360 365 370 375
 Ile Ile Asp His Thr
 380
 <210> 10
 <211> 380
 <212> PRT
 <213> *Geobacillus stearothermophilus*
 <400> 10
 Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr
 1 5 10 15 20 25
 Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys
 30 35 40 45 50
 Arg His Tyr Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn
 55 60 65 70 75
 Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp
 80 85 90 95 100
 His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys
 105 110 115 120 125
 Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp
 130 135 140 145 150
 Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln
 155 160 165 170 175
 Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr
 180 185 190 195 200
 Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu
 205 210 215 220 225

[0009]

Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys
 230 235 240 245 250
 Thr Ile Asn Met Phe Ala Val Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro
 255 260 265 270 275
 Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu
 280 285 290 295 300
 Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp
 305 310 315 320 325
 Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys
 330 335 340 345 350
 Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala
 355 360 365 370 375
 Ile Ile Asp His Ile
 380
 <210> 11
 <211> 380
 <212> PRT
 <213> *Geobacillus stearothermophilus*
 <400> 11
 Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr
 1 5 10 15 20 25
 Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys
 30 35 40 45 50
 Arg His Tyr Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn
 55 60 65 70 75
 Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp
 80 85 90 95 100
 His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Asp Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys
 105 110 115 120 125
 Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp
 130 135 140 145 150
 Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln
 155 160 165 170 175
 Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr
 180 185 190 195 200
 Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu
 205 210 215 220 225
 Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys
 230 235 240 245 250
 Thr Ile Asn Met Phe Ala Val Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro
 255 260 265 270 275
 Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu
 280 285 290 295 300
 Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp

[0010]

305	310	315	320	325
Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys				
330	335	340	345	350
Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala				
355	360	365	370	375
Ile Ile Asp His Lys				
380				
<210> 12				
<211> 380				
<212> PRT				
<213> Geobacillus stearothermophilus				
<400> 12				
Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr				
1 5 10 15 20 25				
Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys				
30 35 40 45 50				
Arg His Phe Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn				
55 60 65 70 75				
Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp				
80 85 90 95 100				
His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys				
105 110 115 120 125				
Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp				
130 135 140 145 150				
Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln				
155 160 165 170 175				
Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr				
180 185 190 195 200				
Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Ile Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu				
205 210 215 220 225				
Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys				
230 235 240 245 250				
Thr Ile Asn Met Phe Ala Leu Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro				
255 260 265 270 275				
Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu				
280 285 290 295 300				
Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp				
305 310 315 320 325				
Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys				
330 335 340 345 350				
Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Asp Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala				
355 360 365 370 375				

[0011]

Ile Ile Asp His Lys
 380
 <210> 13
 <211> 380
 <212> PRT
 <213> *Geobacillus stearothermophilus*
 <400> 13
 Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr
 1 5 10 15 20 25
 Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys
 30 35 40 45 50
 Arg His Tyr Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Ile Phe Asn
 55 60 65 70 75
 Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp
 80 85 90 95 100
 His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys
 105 110 115 120 125
 Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp
 130 135 140 145 150
 Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln
 155 160 165 170 175
 Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr
 180 185 190 195 200
 Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu
 205 210 215 220 225
 Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys
 230 235 240 245 250
 Thr Ile Asn Met Phe Ala Val Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro
 255 260 265 270 275
 Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu
 280 285 290 295 300
 Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp
 305 310 315 320 325
 Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys
 330 335 340 345 350
 Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala
 355 360 365 370 375
 Ile Ile Asp His Lys
 380
 <210> 14
 <211> 380
 <212> PRT
 <213> *Geobacillus stearothermophilus*
 <400> 14

[0012]

Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr
 1 5 10 15 20 25
 Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys
 30 35 40 45 50
 Arg His Phe Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met
 55 60 65 70 75
 Asp Ile Arg Phe His Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn Phe Glu Gln Ala Asp Thr Leu Val Trp
 80 85 90 95 100
 His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys
 105 110 115 120 125
 Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Asp Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp
 130 135 140 145 150
 Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln
 155 160 165 170 175
 Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr
 180 185 190 195 200
 Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu
 205 210 215 220 225
 Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys
 230 235 240 245 250
 Thr Ile Asn Met Phe Ala Val Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro
 255 260 265 270 275
 Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu
 280 285 290 295 300
 Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp
 305 310 315 320 325
 Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys
 330 335 340 345 350
 Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Asp Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala
 355 360 365 370 375
 Ile Ile Asp His Lys
 380

<210> 15

<211> 380

<212> PRT

<213> *Geobacillus stearothermophilus*

<400> 15

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

[0013]

80	85	90	95	100	
His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Thr Met Val Asn Glu Thr Asp Pro Val Lys					
105	110	115	120	125	
Arg Glu Gln Asn Lys Gln Leu Leu Leu LysArg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp					
130	135	140	145	150	
Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln					
155	160	165	170	175	
Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr					
180	185	190	195	200	
Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu					
205	210	215	220	225	
Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys					
230	235	240	245	250	
Thr Ile Asn Met Phe Ala Val Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro					
255	260	265	265	270	
Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu					
275	280	285	290	300	
Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp					
305	310	315	320	325	
Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys					
330	335	340	345	350	
Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala					
355	360	365	370	375	
Ile Ile Asp His Lys					
380					
<210>	16				
<211>	380				
<212>	PRT				
<213>	Geobacillus stearothermophilus				
<400>	16				
Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Lys Ala Pro Gln Leu Asp Gln Arg Tyr					
1	5	10	15	20	25
Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys					
30	35	40	45	50	
Arg His Tyr Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn					
55	60	65	70	75	
Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp					
80	85	90	95	100	
His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys					
105	110	115	120	125	
Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp					
130	135	140	145	150	
Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln					
155	160	165	170	175	

[0014]

Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr
 180 185 190 195 200
 Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu
 205 210 215 220 225
 Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys
 230 235 240 245 250
 Thr Ile Asn Met Phe Ala Val Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro
 255 260 265 270 275
 Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu
 280 285 290 295 300
 Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp
 305 310 315 320 325
 Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys
 330 335 340 345 350
 Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala
 355 360 365 370 375
 Ile Ile Asp His Lys
 380

<210> 17

<211> 380

<212> PRT

<213> *Geobacillus stearothermophilus*

<400> 17

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

[0015]

Thr Ile Asn Met Phe Ala Val Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro
 255 260 265 270 275
 Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu
 280 285 290 295 300
 Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp
 305 310 315 320 325
 Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys
 330 335 340 345 350
 Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala
 355 360 365 370 375
 Ile Ile Asp His Asn
 380
 <210> 18
 <211> 380
 <212> PRT
 <213> *Geobacillus stearothermophilus*
 <400> 18
 Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr
 1 5 10 15 20 25
 Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys
 30 35 40 45 50
 Arg His Phe Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn
 55 60 65 70 75
 Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp
 80 85 90 95 100
 His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys
 105 110 115 120 125
 Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp
 130 135 140 145 150
 Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln
 155 160 165 170 175
 Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr
 180 185 190 195 200
 Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu
 205 210 215 220 225
 Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys
 230 235 240 245 250
 Thr Ile Asn Met Phe Ala Ala Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Met Tyr Gly Trp Pro
 255 260 265 270 275
 Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu
 280 285 290 295 300
 Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp
 305 310 315 320 325
 Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys

[0016]

330	335	340	345	350
Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Leu Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala				
355	360	365	370	375
Ile Ile Asp His Lys				
380				

<210> 19

<211> 380

<212> PRT

<213> Geobacillus stearothermophilus

<400> 19

Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr				
1	5	10	15	20
Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys				
30	35	40	45	50
Arg His Tyr Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn				
55	60	65	70	75
Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp				
80	85	90	95	100
His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys				
105	110	115	120	125
Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp				
130	135	140	145	150
Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln				
155	160	165	170	175
Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr				
180	185	190	195	200
Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu				
205	210	215	220	225
Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys				
230	235	240	245	250
Thr Ile Asn Met Phe Ala Val Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro				
255	260	265	270	275
Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu				
280	285	290	295	300
Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp				
305	310	315	320	325
Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys				

[0017]

330	335	340	345	350
Val Glu Lys Gly Lys Gly Lys Asp Ala	Pro Phe Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala			
355	360	365	370	375
Ile Ile Asp His Lys				
380				
<210>	20			
<211>	380			
<212>	PRT			
<213>	Geobacillus stearothermophilus			
<400>	20			
Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu Asp Gln Arg Tyr				
1	5	10	15	20
Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys				
30	35	40	45	50
Arg His Tyr Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Lys Phe Asn				
55	60	65	70	75
Phe Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp				
80	85	90	95	100
His Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Val Lys				
105	110	115	120	125
Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp				
130	135	140	145	150
Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln				
155	160	165	170	175
Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr				
180	185	190	195	200
Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu				
205	210	215	220	225
Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys				
230	235	240	245	250
Thr Ile Asn Met Phe Ala Val Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro				
255	260	265	270	275
Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu				
280	285	290	295	300
Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp				
305	310	315	320	325
Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys				
330	335	340	345	350
Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Asp Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala				
355	360	365	370	375
Ile Ile Asp His Lys				
380				
<210>	21			
<211>	380			

[0018]

<212> PRT

<213> *Geobacillus stearothermophilus*

<400> 21

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

Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp
 305 310 315 320 325
 Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys
 330 335 340 345 350
 Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Phe Val Phe Asp Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala
 355 360 365 370 375
 Ile Ile Asp His Lys
 380

<210> 22

<211> 380

<212> PRT

<213> *Geobacillus stearothermophilus*

<400> 22

Met Lys Asn Ala Asp Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Lys Ala Pro Gln Leu Asp Gln Arg Tyr Ile
 1 5 10 15 20 25
 Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys Arg

[0019]

30	35	40	45	50
His Tyr Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro Glu Glu Gly Ile Phe Asn Phe				
55	60	65	70	75
Glu Gln Ala Asp Arg Ile Val Lys Phe Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp His				
80	85	90	95	100
Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Asp Gly Lys Pro Met Val Asn Glu Ile Asp Pro Val Lys Arg				
105	110	115	120	125
Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Asp Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys Asp				
130	135	140	145	150
Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln				
155	160	165	170	175
Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile Lys Leu Tyr				
180	185	190	195	200
Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr Ala Leu Tyr Asn Leu Ala Lys Gln Leu Lys Glu Glu				
205	210	215	220	225
Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys				
230	235	240	245	250
Thr Ile Asn Met Phe Ala Val Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Ile Tyr Gly Trp Pro				
255	260	265	270	275
Pro Arg Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu				
280	285	290	295	300
Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp				
305	310	315	320	325
Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala Lys				
330	335	340	345	350
Val Glu Lys Gly Lys Gly Lys Asp Ala Pro Leu Val Phe Asp Pro Asp Tyr Lys Val Lys Pro Ala Tyr Trp Ala				
355	360	365	370	375
Ile Ile Asp His Asn				
380				

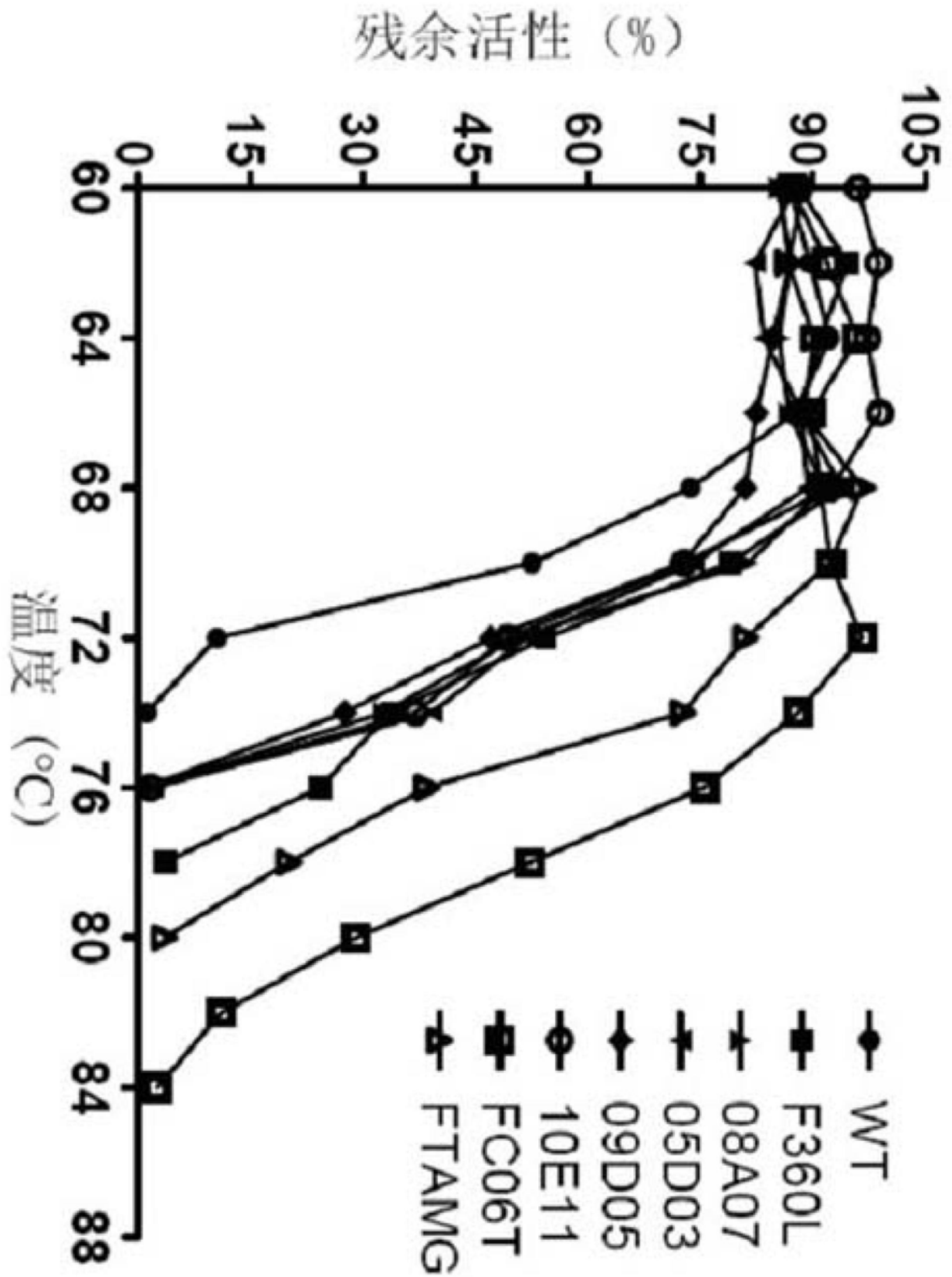


图 1

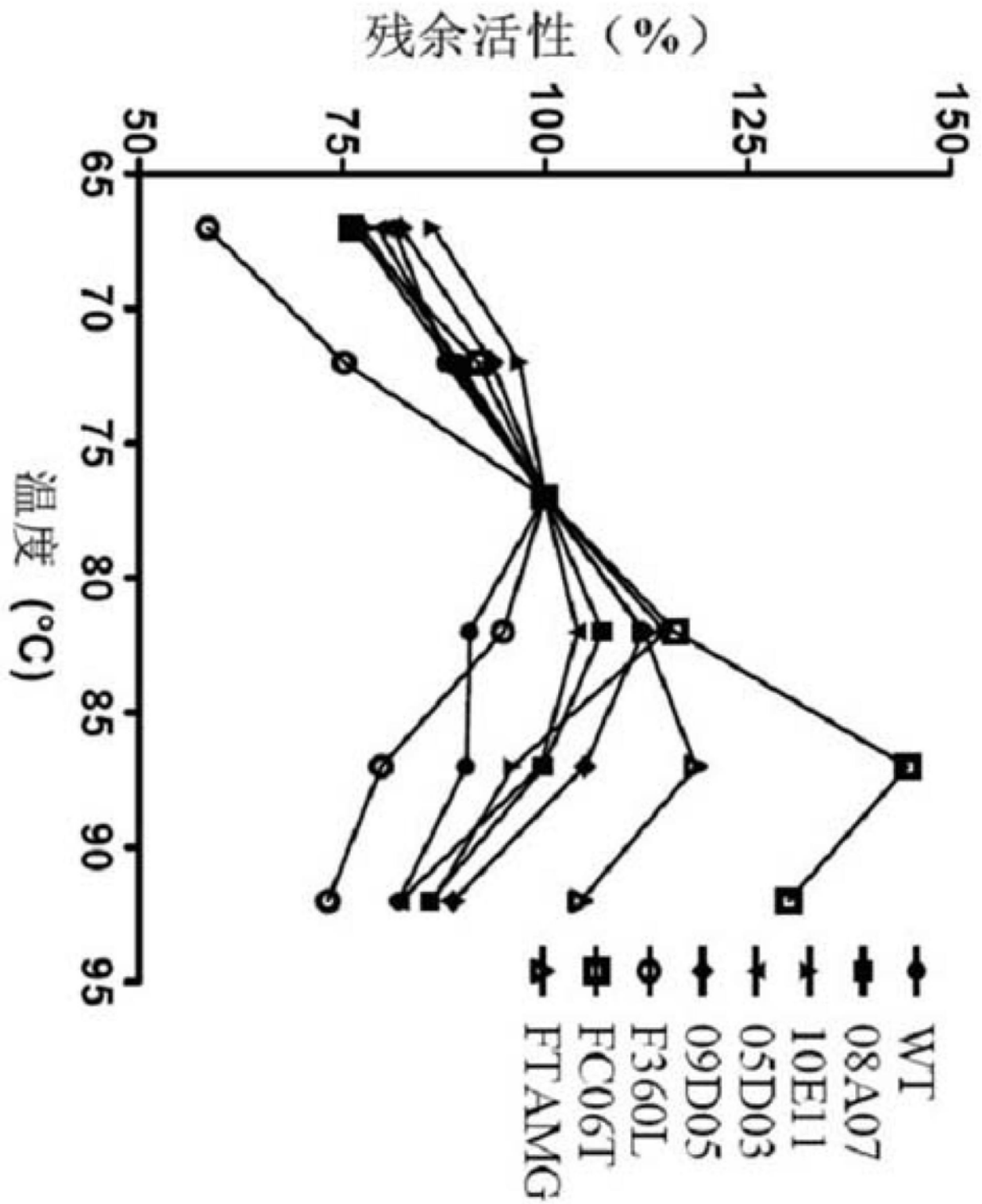


图 2