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CN 105154462 A,2015.12.16
阮瑶等.大肠埃希菌CLM37菌株Lpp基因敲除及胞外表达N-糖基化重组蛋白研究.《上海交通大学学报(医学版)》.2018,(第11期),摘要.

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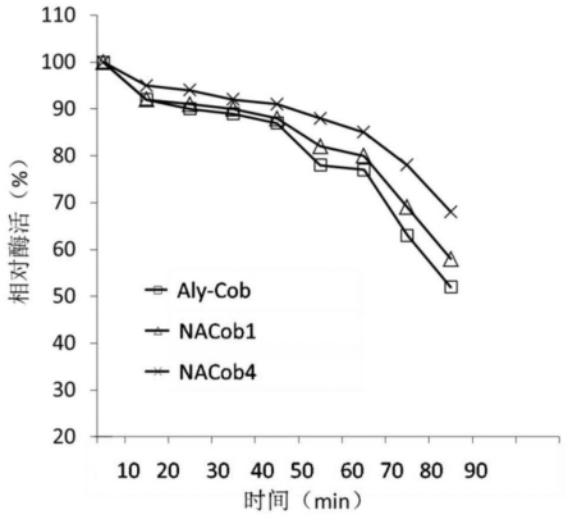
(54)发明名称

一种可高效应用的N-糖基化褐藻胶裂解酶突变体及基因工程菌构建方法

(57)摘要

本发明公开了一种可高效应用的N-糖基化褐藻胶裂解酶突变体及基因工程菌构建方法,属于生物技术领域。本发明是在褐藻胶裂解酶Aly-Cob的基础上,在其N端引入DQNAT重复序列片段作为N-糖基化位点,同时融合表达载体蛋白YebF进行胞外分泌,进而由空肠弯曲杆菌来源的糖基化体系pgl以糖链$GalNAc_2(Glc)GalNAc_3$Bac进行糖基化修饰。所得突变体酶NACob在酶活及稳定性上均较糖基化前得以提高。本发明提供的N-糖基化修饰褐藻胶裂解酶突变体可广泛应用于海藻加工利用相关的食品、医药、农业等领域。



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1.一种N-糖基化褐藻胶裂解酶突变体的构建方法,具有如下步骤:

(1)质粒pTrc99A用XbaI与HindIII双酶切,引入编码糖基化位点DQNAT及6×His基因序列,构建重组质粒,经XbaI与SalI双酶切后,插入NCBI登录号为B1847的YebF基因序列,形成pTrc-YebF-gly-His质粒,再经XbaI和EcoR I双酶切后,插入Aly-Cob编码基因,形成重组载体pTrc-YebF-gly-Aly-Cob-His;

(2)将构建的含褐藻胶裂解酶基因序列的表达质粒与携带空肠弯曲菌N-糖基化基因簇的pACYCpgl质粒,电击转化至大肠杆菌进行共表达,转化子接种到含有20μg/mL氯霉素和100μg/mL甲氧苄啶的LB培养基中培养16-20h后,添加0.01mM异丙基硫代半乳糖苷诱导表达24h;

(3)离心收集发酵上清,通过镍柱子纯化重组酶;

所述重组载体pTrc-YebF-gly-Aly-Cob-His表达氨基酸序列如SEQ ID No.1或SEQ ID No.2所示的融合蛋白。

2.权利要求1所述的方法所产褐藻胶裂解酶在褐藻寡糖制备中的应用。

一种可高效应用的N-糖基化褐藻胶裂解酶突变体及基因工程菌构建方法

技术领域

[0001] 本发明涉及一种糖基化修饰的海洋褐藻酸裂解酶及其应用，属于生物技术领域。本发明提供的褐藻胶裂解酶可广泛应用于海藻加工利用的食品、医药、农业等领域。

背景技术

[0002] 褐藻寡糖是褐藻胶经降解产生的一类具有抗肿瘤、免疫调节、降血糖血脂等多种生物活性的高附加值低聚糖，在食品、医药、饲料等领域具有重要应用。以褐藻胶裂解酶作为工具酶酶解制备褐藻寡糖的过程具有反应条件温和、过程可控、得率高等优点，已逐渐成为制备褐藻寡糖的主要方式。

[0003] 目前，已经从海洋动植物及环境样品中分离获得各类产褐藻胶裂解酶的微生物，种类丰富且性能多样。因从海洋来源样品中获取，大部分产酶微生物具有生长条件特殊、产酶量低等特点，大大限制了酶的发展和应用。为解决目前褐藻胶裂解酶存在的缺陷，已有多个产酶基因通过基因工程菌的构建，实现了外源表达，培养条件得以简化，且产酶量有所提升。但普遍仍存在表达水平较低、酶活不高等问题。需要进一步选择或改进酶的改造方法以进一步提升酶的应用潜力。

[0004] 糖基化是生物体内广泛存在与发生的蛋白质翻译后修饰过程，包括了N位糖基化、O位糖基化、C位甘露糖化以及GPI(glycophosphatidlyinositol)锚定连接，尤以N位糖基化最为普遍。研究表明，N位糖基化对酶的结构、功能、稳定性、相互作用等方面具有显著影响，是一种有效的人工进行酶后修饰的手段，在酶的改造、蛋白药物、抗体工程等各个领域均有广泛应用。近年来，随着对空肠弯曲杆菌(Campylobacter jejuni)蛋白N-糖基化修饰研究的不断深入，利用大肠杆菌重建原核糖基化系统为酶的糖基化修饰和糖蛋白的可控制备提供了条件。大肠杆菌具有遗传背景清晰、生长快、操作简单等特点，是酶的表达和改造的理想宿主。利用大肠杆菌体系进行酶的糖基化修饰，提升酶的催化性能，改善酶的稳定性，对于酶的改造及其应用具有重要的理论研究价值和应用前景。

发明内容

[0005] 本发明的目的是提供一种能高效应用的N-糖基化褐藻胶裂解酶突变体及基因工程菌构建方法。

[0006] 所述突变体，是在褐藻胶裂解酶Aly-Cob的基础上，在其N端引入载体蛋白YebF进行融合表达，同时引入n×DQNAT重复序列片段(n为1～10)作为N-糖基化位点，与来源空肠弯曲菌糖基化修饰体系pgl基因簇共表达，形成以糖链$GalNAc_2(Glc)GalNAc_3Bac$修饰的突变体NACob。

[0007] 所述突变体，在本发明的一种实施方式中，是在Aly-Cob的N端引入载体蛋白YebF与1×DQNAT序列，突变经修饰得到的褐藻胶裂解酶命名为NACob1，其氨基酸序列如SEQ ID No.1所示。

[0008] SEQ ID No.1：

1 MET Lys Lys Arg Gly Ala Phe Leu Gly Leu Leu Leu Val Ser Ala

16 Cys Ala Ser Val Phe Ala Ala Asn Asn Glu Thr Ser Lys Ser Val

[0009] 31 Thr Phe Pro Lys Cys Glu Asp Leu Asp Ala Ala Gly Ile Ala Ala

46 Ser Val Lys Arg Asp Tyr Gln Gln Asn Arg Val Ala Arg Trp Ala

61 Asp Asp Gln Lys Ile Val Gly Gln Ala Asp Pro Val Ala Trp Val

76 Ser Leu Gln Asp Ile Gln Gly Lys Asp Asp Lys Trp Ser Val Pro

91 Leu Thr Val Arg Gly Lys Ser Ala Asp Ile His Tyr Gln Val Ser

106 Val Asp Cys Lys Ala Gly MET Ala Glu Tyr Gln Arg Arg Asp Gln

121 Asn Ala Thr Asn Asp Thr Pro Pro Gly Glu Thr Phe Asp Leu Asp

136 Thr Trp Lys Leu Thr Leu Pro MET Asp Ala Asp Gly Asn Gly Lys

151 Val Asp Glu Ile Lys Val Ala Asp Leu Gln Ser Tyr Arg His Ser

166 Asp Tyr Phe Tyr Leu Asp Asp Asp Ser His MET Val Phe Val Thr

181 Pro Asn Lys Ala Phe Thr Thr Pro Asn Ser Ser Asn Ala Arg Thr

196 Glu Leu Arg Gln MET Leu Arg Gly Thr Asp Thr Ser Ile Gly Thr

211 His Asp Pro Lys Asn Asn Phe Ala Leu Ala Ser Asn Gln His Ala

226 Asp Glu Phe Ala Gln Ile Gly Gly Tyr Leu Ser Ala Thr Leu Arg

241 Val Glu His Val Ala Glu Arg Ser Lys Lys Pro Asp Arg Lys Ser

[0010] 256 Ala Tyr Ser Val Val Val Gly Gln Ile His Ala Gly Lys Asp Gln

271 Ala Leu MET Glu Ala Asp Glu Gly Phe Gly His Gly Asn Glu Pro

286 Leu Lys Ile Phe Tyr Lys Lys Leu Pro Asp Asp Lys Thr Gly Ser

301 Val Phe Trp Asn Tyr Glu Lys Asn Leu Ala Lys Glu Asp Pro Lys

316 Arg Thr Asp Val Ser Tyr Ala Val Trp Gly Asn Asp Trp Ser Ser

331 Asn Ala Asp Pro Gly Lys Glu Gly Ile Ala Leu Gly Asp Thr Phe

346 Ser Tyr Lys Val Glu Val Lys Gly Asp Ile MET His Leu Thr Phe

361 Asn Ala Asp Gly His Pro Thr His Asn Phe Glu Ile Asn Leu Ala

376 Asp Asn Val Asp Ala Asn Gly Lys Val Asp Asn Asp Asp Leu Pro

391 Ala Gly Tyr Ala Gly Asp Trp MET Tyr Phe Lys Ala Gly Ser Tyr

406 Asn Gln Cys Asn Thr Lys Ala Ser Ser Asn Ala Cys Glu Gly Thr

421 Gly Val Trp Glu Thr Asp Lys Ala Asn Gly Asp Tyr Ala Lys Val

436 Val Phe Thr Lys Val Glu Ser Gly Glu MET Gln His His His His

452 His His

[0011] 所述突变体，在本发明的一种实施方式中，是在Aly-Cob的N端引入载体蛋白YebF与4×DQNAT序列，突变经修饰得到的褐藻胶裂解酶命名为NACob4，其氨基酸序列如SEQ ID No.2所示。

[0012] SEQ ID No.2：

1	MET Lys Lys Arg Gly Ala Phe Leu Gly Leu Leu Leu Val Ser Ala
16	Cys Ala Ser Val Phe Ala Ala Asn Asn Glu Thr Ser Lys Ser Val
31	Thr Phe Pro Lys Cys Glu Asp Leu Asp Ala Ala Gly Ile Ala Ala

[0013]

46	Ser Val Lys Arg Asp Tyr Gln Gln Asn Arg Val Ala Arg Trp Ala
61	Asp Asp Gln Lys Ile Val Gly Gln Ala Asp Pro Val Ala Trp Val
76	Ser Leu Gln Asp Ile Gln Gly Lys Asp Asp Lys Trp Ser Val Pro

[0014]

91	Leu Thr Val Arg Gly Lys Ser Ala Asp Ile His Tyr Gln Val Ser
106	Val Asp Cys Lys Ala Gly MET Ala Glu Tyr Gln Arg Arg Asp Gln
121	Asn Ala Thr Asp Gln Asn Ala Thr Asp Gln Asn Ala Thr Asp Gln
136	Asn Ala Thr Asn Asp Thr Pro Pro Gly Glu Thr Phe Asp Leu Asp
151	Thr Trp Lys Leu Thr Leu Pro MET Asp Ala Asp Gly Asn Gly Lys
166	Val Asp Glu Ile Lys Val Ala Asp Leu Gln Ser Tyr Arg His Ser
181	Asp Tyr Phe Tyr Leu Asp Asp Asp Ser His MET Val Phe Val Thr
196	Pro Asn Lys Ala Phe Thr Thr Pro Asn Ser Ser Asn Ala Arg Thr
211	Glu Leu Arg Gln MET Leu Arg Gly Thr Asp Thr Ser Ile Gly Thr
226	His Asp Pro Lys Asn Asn Phe Ala Leu Ala Ser Asn Gln His Ala
241	Asp Glu Phe Ala Gln Ile Gly Gly Tyr Leu Ser Ala Thr Leu Arg
256	Val Glu His Val Ala Glu Arg Ser Lys Lys Pro Asp Arg Lys Ser
271	Ala Tyr Ser Val Val Val Gly Gln Ile His Ala Gly Lys Asp Gln
286	Ala Leu MET Glu Ala Asp Glu Gly Phe Gly His Gly Asn Glu Pro
301	Leu Lys Ile Phe Tyr Lys Lys Leu Pro Asp Asp Lys Thr Gly Ser
316	Val Phe Trp Asn Tyr Glu Lys Asn Leu Ala Lys Glu Asp Pro Lys
331	Arg Thr Asp Val Ser Tyr Ala Val Trp Gly Asn Asp Trp Ser Ser
346	Asn Ala Asp Pro Gly Lys Glu Gly Ile Ala Leu Gly Asp Thr Phe
361	Ser Tyr Lys Val Glu Val Lys Gly Asp Ile MET His Leu Thr Phe
376	Asn Ala Asp Gly His Pro Thr His Asn Phe Glu Ile Asn Leu Ala
391	Asp Asn Val Asp Ala Asn Gly Lys Val Asp Asn Asp Asp Leu Pro
406	Ala Gly Tyr Ala Gly Asp Trp MET Tyr Phe Lys Ala Gly Ser Tyr
421	Asn Gln Cys Asn Thr Lys Ala Ser Ser Asn Ala Cys Glu Gly Thr
436	Gly Val Trp Glu Thr Asp Lys Ala Asn Gly Asp Tyr Ala Lys Val
451	Val Phe Thr Lys Val Glu Ser Gly Glu MET Gln His His His His
467	His His

[0015] 所述Cobetia sp.来源褐藻胶裂解酶，发生N-糖基化突变前的氨基酸序列如SEQ ID No.2所示，由于不同Cobetia sp.菌株来源的褐藻胶裂解酶序列仅有个别氨基酸的差异，因此，该方法不仅适用于SEQ ID No.2序列，且对氨基酸序列相似性达到80％以上的褐藻胶裂解酶序列均适用于本专利。

[0016] 所述表达载体为pET28a(+)、pET24b、pACYC、pMAF10、pSP73其中的一种。

[0017] 所述基因工程菌是以大肠杆菌为宿主构建的,可以是E.coli BL21(DE3)、CLM24、CLM37中的任意一种。

[0018] 所述基因工程菌,在本发明的一种实施方式中,其构建方法是:将质粒pTrc与载体蛋白YebF及糖基化DQNAT重复序列片段构建重组质粒,酶切后连接编码所述褐藻胶裂解酶的核苷酸序列,与含糖基化体系的pACYCpgl质粒共同电转入E.coli BL21(DE3)感受态细胞中进行表达与糖基化。

[0019] 本发明提供了一种提高褐藻胶裂解酶稳定性的方法,是将褐藻胶裂解酶N端进行N-糖基化突变和修饰。

[0020] 本发明的有益效果:

[0021] 本发明的经过N-糖基化突变修饰的褐藻胶裂解酶,酶的表达与菌体生长不受N-糖基化影响。发酵培养24h后,酶活提高7%以上,40℃下温度稳定性提高了15%以上。

[0022] 本发明的褐藻胶裂解酶可广泛用于海藻加工相关的食品、医药、化工、农业等领域。

附图说明

[0023] 图1:糖基化前后褐藻胶裂解酶的温度稳定性对比

具体实施方式

[0024] 实施例1:引入N-糖基化位点的突变酶NACob1及工程菌构建

[0025] 本发明在褐藻胶裂解酶Aly-Cob的N端设计引入N-糖基化位点1×DQNAT,并与载体蛋白YebF融合表达,经与糖基化pglB质粒共表达后,产生N-糖基化修饰改造的褐藻胶裂解酶NACob1(氨基酸序列如SEQ ID NO.1)。

[0026] 具体方法如下:

[0027] (1)将质粒pTrc99A用XbaI与HindIII双酶切,引入编码糖基化位点DQNAT及6×His基因序列,构建重组质粒,经XbaI与SalI双酶切后,插入YebF(NCBI:B1847)基因序列,形成pTrc-YebF-gly-His质粒,经XbaI和EcoR I双酶切后,插入Aly-Cob编码基因,形成重组载体pTrc-YebF-gly-Aly-Cob-His;

[0028] (2)将构建的表达质粒与携带空肠弯曲菌N-糖基化基因簇的pACYCpgl质粒,电击转化至大肠杆菌E.coli CLM24共表达,然后将转化子接种到含有氯霉素(20μg/mL)和甲氧苄啶(100μg/mL)的LB培养基中,培养16-20h后,添加异丙基硫代半乳糖苷(IPTG,0.01mM)诱导表达24h;

[0029] (3)离心收集发酵上清,通过镍柱子纯化重组酶NACob1。

[0030] 实施例2:引入N-糖基化位点的突变酶NACob4及工程菌构建

[0031] 方法与实施例1类似,不同在于糖基化位点为4×DQNAT,所得突变酶命名为NACob4(氨基酸序列如SEQ ID NO.2)。

[0032] 实施例3:糖基化修饰前后酶活的比较

[0033] 采用DNS法测定发酵上清中的褐藻胶裂解酶活性(表1)。糖基化修饰后,褐藻胶裂解酶的活力得以少量提升,酶活NACob4>NACob>Aly-Cob,其中NACob4酶活提高了12.6%。

[0034] 表1糖基化修饰前后褐藻胶裂解酶酶活

[0035]

	酶活（U/mL）	相对酶活（%）
Aly-Cob	401.3±0.9	100
NACob1	430.6±1.4	107.3
NACob4	452.0±1.1	112.6

[0036] 实施例4:糖基化修饰前后酶稳定性的比较

[0037] 将糖基化修饰前后的酶液分别在40℃保温30min,测定残留的酶活力,将最高酶活力定义为100%。每10min取样测定样品残余酶活力。最终确定糖基化修饰后,褐藻胶裂解酶的稳定性得到提高,尤其是NACob4,其40℃稳定性比Aly-Cob提高了26%(图1)。

[0001] 序列表

[0002] <110> 江南大学

[0003] <120> 一种可高效应用的N-糖基化褐藻胶裂解酶突变体及基因工程菌构建方法

[0004] <140> 2019101597811

[0005] <141> 2019-03-04

[0006] <160> 2

[0007] <170> SIPOSequenceListing 1.0

[0008] <210> 1

[0009] <211> 452

[0010] <212> PRT

[0011] <213> 人工序列(Artificial Sequence)

[0012] <400> 1

[0013] Met Lys Lys Arg Gly Ala Phe Leu Gly Leu Leu Leu Val Ser Ala Cys

[0014] 1 5 10 15

[0015] Ala Ser Val Phe Ala Ala Asn Asn Glu Thr Ser Lys Ser Val Thr Phe

[0016] 20 25 30

[0017] Pro Lys Cys Glu Asp Leu Asp Ala Ala Gly Ile Ala Ala Ser Val Lys

[0018] 35 40 45

[0019] Arg Asp Tyr Gln Gln Asn Arg Val Ala Arg Trp Ala Asp Asp Gln Lys

[0020] 50 55 60

[0021] Ile Val Gly Gln Ala Asp Pro Val Ala Trp Val Ser Leu Gln Asp Ile

[0022] 65 70 75 80

[0023] Gln Gly Lys Asp Asp Lys Trp Ser Val Pro Leu Thr Val Arg Gly Lys

[0024] 85 90 95

[0025] Ser Ala Asp Ile His Tyr Gln Val Ser Val Asp Cys Lys Ala Gly Met

[0026] 100 105 110

[0027] Ala Glu Tyr Gln Arg Arg Asp Gln Asn Ala Thr Asn Asp Thr Pro Pro

[0028] 115 120 125

[0029] Gly Glu Thr Phe Asp Leu Asp Thr Trp Lys Leu Thr Leu Pro Met Asp

[0030] 130 135 140

[0031] Ala Asp Gly Asn Gly Lys Val Asp Glu Ile Lys Val Ala Asp Leu Gln

[0032] 145 150 155 160

[0033] Ser Tyr Arg His Ser Asp Tyr Phe Tyr Leu Asp Asp Asp Ser His Met

[0034] 165 170 175

[0035] Val Phe Val Thr Pro Asn Lys Ala Phe Thr Thr Pro Asn Ser Ser Asn

[0036] 180 185 190

[0037] Ala Arg Thr Glu Leu Arg Gln Met Leu Arg Gly Thr Asp Thr Ser Ile

[0038] 195 200 205

[0039] Gly Thr His Asp Pro Lys Asn Asn Phe Ala Leu Ala Ser Asn Gln His
[0040] 210 215 220
[0041] Ala Asp Glu Phe Ala Gln Ile Gly Gly Tyr Leu Ser Ala Thr Leu Arg
[0042] 225 230 235 240
[0043] Val Glu His Val Ala Glu Arg Ser Lys Lys Pro Asp Arg Lys Ser Ala
[0044] 245 250 255
[0045] Tyr Ser Val Val Val Gly Gln Ile His Ala Gly Lys Asp Gln Ala Leu
[0046] 260 265 270
[0047] Met Glu Ala Asp Glu Gly Phe Gly His Gly Asn Glu Pro Leu Lys Ile
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[0050] 290 295 300
[0051] Tyr Glu Lys Asn Leu Ala Lys Glu Asp Pro Lys Arg Thr Asp Val Ser
[0052] 305 310 315 320
[0053] Tyr Ala Val Trp Gly Asn Asp Trp Ser Ser Asn Ala Asp Pro Gly Lys
[0054] 325 330 335
[0055] Glu Gly Ile Ala Leu Gly Asp Thr Phe Ser Tyr Lys Val Glu Val Lys
[0056] 340 345 350
[0057] Gly Asp Ile Met His Leu Thr Phe Asn Ala Asp Gly His Pro Thr His
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[0059] Asn Phe Glu Ile Asn Leu Ala Asp Asn Val Asp Ala Asn Gly Lys Val
[0060] 370 375 380
[0061] Asp Asn Asp Asp Leu Pro Ala Gly Tyr Ala Gly Asp Trp Met Tyr Phe
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[0063] Lys Ala Gly Ser Tyr Asn Gln Cys Asn Thr Lys Ala Ser Ser Asn Ala
[0064] 405 410 415
[0065] Cys Glu Gly Thr Gly Val Trp Glu Thr Asp Lys Ala Asn Gly Asp Tyr
[0066] 420 425 430
[0067] Ala Lys Val Val Phe Thr Lys Val Glu Ser Gly Glu Met Gln His His
[0068] 435 440 445
[0069] His His His His
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[0071] <210> 2
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[0073] <212> PRT
[0074] <213> 人工序列(Artificial Sequence)
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[0076] Met Lys Lys Arg Gly Ala Phe Leu Gly Leu Leu Leu Val Ser Ala Cys
[0077] 1 5 10 15

[0078] Ala Ser Val Phe Ala Ala Asn Asn Glu Thr Ser Lys Ser Val Thr Phe
[0079] 20 25 30
[0080] Pro Lys Cys Glu Asp Leu Asp Ala Ala Gly Ile Ala Ala Ser Val Lys
[0081] 35 40 45
[0082] Arg Asp Tyr Gln Gln Asn Arg Val Ala Arg Trp Ala Asp Asp Gln Lys
[0083] 50 55 60
[0084] Ile Val Gly Gln Ala Asp Pro Val Ala Trp Val Ser Leu Gln Asp Ile
[0085] 65 70 75 80
[0086] Gln Gly Lys Asp Asp Lys Trp Ser Val Pro Leu Thr Val Arg Gly Lys
[0087] 85 90 95
[0088] Ser Ala Asp Ile His Tyr Gln Val Ser Val Asp Cys Lys Ala Gly Met
[0089] 100 105 110
[0090] Ala Glu Tyr Gln Arg Arg Asp Gln Asn Ala Thr Asp Gln Asn Ala Thr
[0091] 115 120 125
[0092] Asp Gln Asn Ala Thr Asp Gln Asn Ala Thr Asn Asp Thr Pro Pro Gly
[0093] 130 135 140
[0094] Glu Thr Phe Asp Leu Asp Thr Trp Lys Leu Thr Leu Pro Met Asp Ala
[0095] 145 150 155 160
[0096] Asp Gly Asn Gly Lys Val Asp Glu Ile Lys Val Ala Asp Leu Gln Ser
[0097] 165 170 175
[0098] Tyr Arg His Ser Asp Tyr Phe Tyr Leu Asp Asp Asp Ser His Met Val
[0099] 180 185 190
[0100] Phe Val Thr Pro Asn Lys Ala Phe Thr Thr Pro Asn Ser Ser Asn Ala
[0101] 195 200 205
[0102] Arg Thr Glu Leu Arg Gln Met Leu Arg Gly Thr Asp Thr Ser Ile Gly
[0103] 210 215 220
[0104] Thr His Asp Pro Lys Asn Asn Phe Ala Leu Ala Ser Asn Gln His Ala
[0105] 225 230 235 240
[0106] Asp Glu Phe Ala Gln Ile Gly Gly Tyr Leu Ser Ala Thr Leu Arg Val
[0107] 245 250 255
[0108] Glu His Val Ala Glu Arg Ser Lys Lys Pro Asp Arg Lys Ser Ala Tyr
[0109] 260 265 270
[0110] Ser Val Val Val Gly Gln Ile His Ala Gly Lys Asp Gln Ala Leu Met
[0111] 275 280 285
[0112] Glu Ala Asp Glu Gly Phe Gly His Gly Asn Glu Pro Leu Lys Ile Phe
[0113] 290 295 300
[0114] Tyr Lys Lys Leu Pro Asp Asp Lys Thr Gly Ser Val Phe Trp Asn Tyr
[0115] 305 310 315 320
[0116] Glu Lys Asn Leu Ala Lys Glu Asp Pro Lys Arg Thr Asp Val Ser Tyr

[0117] 325 330 335

[0118] Ala Val Trp Gly Asn Asp Trp Ser Ser Asn Ala Asp Pro Gly Lys Glu

[0119] 340 345 350

[0120] Gly Ile Ala Leu Gly Asp Thr Phe Ser Tyr Lys Val Glu Val Lys Gly

[0121] 355 360 365

[0122] Asp Ile Met His Leu Thr Phe Asn Ala Asp Gly His Pro Thr His Asn

[0123] 370 375 380

[0124] Phe Glu Ile Asn Leu Ala Asp Asn Val Asp Ala Asn Gly Lys Val Asp

[0125] 385 390 395 400

[0126] Asn Asp Asp Leu Pro Ala Gly Tyr Ala Gly Asp Trp Met Tyr Phe Lys

[0127] 405 410 415

[0128] Ala Gly Ser Tyr Asn Gln Cys Asn Thr Lys Ala Ser Ser Asn Ala Cys

[0129] 420 425 430

[0130] Glu Gly Thr Gly Val Trp Glu Thr Asp Lys Ala Asn Gly Asp Tyr Ala

[0131] 435 440 445

[0132] Lys Val Val Phe Thr Lys Val Glu Ser Gly Glu Met Gln His His His

[0133] 450 455 460

[0134] His His His

[0135] 465

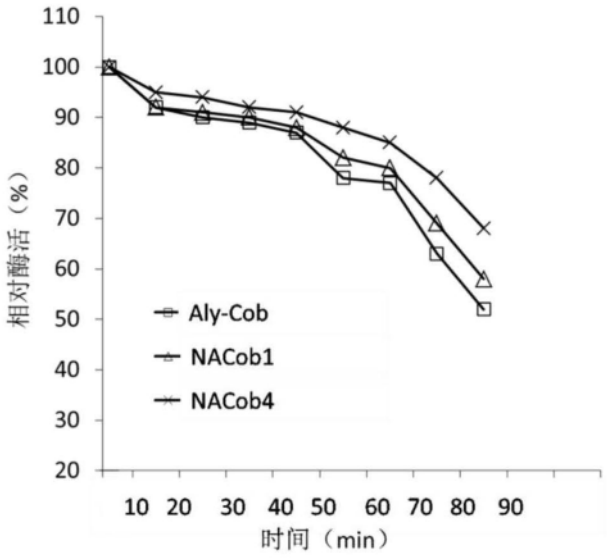
110
100
90
80
70
60
50
40
30
20
相对酶活（%）
Aly-Cob
NACob1
NACob4
10 20 30 40 50 60 70 80 90
时间（min）

图1