I627153

(19)中華民國智慧財產局

(12)發明說明書公告本 **(11)證書號數：TW I627153 B**

(45)公告日： 中華民國 107 (2018) 年 06 月 21 日

(21)申請案號：104120521 (22)申請日： 中華民國 98 (2009) 年 03 月 11 日

(51)Int. Cl.： *C07C229/08 (2006.01)* *C12P13/00 (2006.01)*
C12P17/10 (2006.01)

(30)優先權：2008/03/11 歐洲專利局 08152584.2

(71)申請人：ＤＳＭ智慧財產有限公司 (荷蘭) DSM IP ASSETS B. V. (NL)
荷蘭

(72)發明人：雷瑪克斯 法蘭肯 佩桌妮娜C RAEMAKERS-FRANKEN, PETRONELLA CATHARINA (NL)；休曼 馬汀 SCHURMANN, MARTIN (DE)；崔夫瑟 艾克索C TREFZER, AXEL CHRISTOPH (DE)；迪維爾德曼 史蒂芬M A DE WILDEMAN, STEFAAN MARIE ANDRE (BE)

(74)代理人：惲軼群；陳文郎

(56)參考文獻：

TW 201033369A WO 2005/068643A2

審查人員：簡正芳

申請專利範圍項數：12 項 圖式數：0 共 173 頁

(54)名稱

由 α-酮庚二酸製備 6-胺己酸之技術

PREPARATION OF 6-AMINOCAPROIC ACID FROM α-KETOPIMELIC ACID

(57)摘要

本發明係關於一種使用生物催化劑製備 6-胺己酸(後文也稱作為「6-ACA」)之方法。本發明進一步係關於一種經由環化此種 6-ACA 製備 ε-己內醯胺(後文稱作為「己內醯胺」)之方法。本發明進一步係關於可用於 6-ACA 或己內醯胺之製備之宿主細胞、微生物、或多核苷酸。

The invention relates to a method for preparing 6-aminocaproic acid (hereinafter also referred to as '6-ACA') using a biocatalyst. The invention further relates to a method for preparing ε-caprolactam (hereafter referred to as 'caprolactam') by cyclising such 6-ACA. The invention further relates to a host cell, a micro-organism, or a polynucleotide which may be used in the preparation of 6-ACA or caprolactam.

發明專利說明書

（本說明書格式、順序，請勿任意更動）

【發明名稱】（中文/英文）

由α-酮庚二酸製備6-胺己酸之技術

PREPARATION OF 6-AMINOCAPROIC ACID FROM α-KETOPIMELIC ACID

【技術領域】

發明領域

[0001]本發明係關於一種製備6-胺己酸(後文也稱作爲「6-ACA」)之方法。本發明進一步係關於一種由6-ACA製備ε-己內醯胺(後文稱作爲「己內醯胺」)之方法。本發明進一步係關於可用於6-ACA或己內醯胺之製備之宿主細胞。

【先前技術】

發明背景

[0002]己內醯胺爲可用於聚醯胺例如尼龍-6或尼龍-6,12 (己內醯胺與月桂內醯胺之共聚物)之製造之內醯胺。技藝界已知多種由散裝化學品製備己內醯胺之方式，包括由環己酮、甲苯、酚、環己醇、苯或環己烷製備己內醯胺之方式。此等中間化合物通常係得自礦油。有鑑於對使用更加綠色的永續性技術製備材料之需求的成長，期望提供一種方法其中己內醯胺係由一種得自生物可再生來源之中間化合物製備，或己內醯胺至少係得自可使用生物化學方法而轉化成爲己內醯胺之中間化合物。進一步期望提供一種比較習知利用得自石化來源的散裝化學品之化學方法需要更少能源之方法。

[0003]已知由6-ACA製備己內醯胺，例如如US-A 6,194,572所述。如揭示於WO 2005/068643，經由於具有α,β-烯酸還原酶活性之酶存在下，經由轉化6-胺己-2-烯酸(6-AHEA)可藉生物化學方式製備6-ACA。6-AHEA例如可以生物化學方式或藉純化學合成法而由離胺酸製備。雖然藉WO 2005/068643所揭示之方法透過6-AHEA之還原製備6-ACA爲可行，但發明人發現於還原反應條件下，6-AHEA可能自發地且實質上不可逆地環化而形成非期望的副產物，值得注意者爲β-高脯胺酸。此種環化作用可能成爲6-ACA製造上的瓶頸，且可能導致產率上的顯著損耗。

【發明內容】

發明概要

[0004]本發明之目的係提供一種用於製備6-ACA或己內醯胺之新穎方法，該方法可用於聚醯胺或6-ACA或己內醯胺製備上的中間物之製備，而可用作爲已知方法之替代方法。

[0005]又一目的係提供一種可克服前述一項或多項缺點之新穎方法。

[0006]根據本發明可解決之一項或多項其它目的由後文說明獲得。

[0007]今日已知由特定起始物料製備6-ACA，換言之，發現可製備6-胺己酸(6-ACA)，其中6-胺己酸係由2-酮庚二酸也稱作爲α-酮庚二酸(AKP)製備。特定言之，該方法可於兩個或多個反應步驟進行。例如提供一種方法，其中AKP

首先轉成5-甲醯戊酸酯(5-甲醯戊酸，5-FVA)，該5-FVA轉成6-ACA。進一步提供一種方法，其中AKP首先轉成α-胺庚二酸(AAP)。隨後AAP轉成6-ACA。

[0008]發明人瞭解原則上可以全然化學(換言之未使用生物催化劑)方式而由AKP製備6-ACA。進行個別反應步驟之適當化學方式之實例列舉如下。但發明人也瞭解可由AKP以生物化學方式製備6-ACA。

[0009]如此，本發明特別係關於一種用於製備6-ACA之方法，其中6-ACA係使用至少一種生物催化劑而由AKP製備。

[0010]本發明進一步係關於一種方法，其中6-ACA係使用生物催化劑而由5-甲醯戊酸酯(5-甲醯戊酸，5-FVA)製備。如前文指示，5-FVA可得自AKP。

[0011]於一個實施例中，於本發明方法所製備之6-ACA可用於製備己內醯胺。此種方法包含視需要可於生物催化劑存在下，環化6-胺己酸。

【圖式簡單說明】

(無)

【實施方式】

較佳實施例之詳細說明

[0012]於本文中述及羧酸或羧酸酯例如6-ACA、2-胺庚二酸(α-胺庚二酸，後文也縮寫爲「AAP」)、其它胺基酸、5-FVA或AKP時，此等術語表示包括經質子化之羧酸基(亦即中性基)、其相對應之羧酸根(其軛合鹼)及其鹽。當於本

文述及胺基酸例如6-ACA時，本術語表示包括呈其兩性離子形式之胺基酸(其中胺基係呈質子化形式及羧酸基係呈去質子化形式)、其中胺基經質子化及羧酸呈其中性形式之胺基酸、及其中胺基係呈其中性形式及羧酸基係呈去質子化形式之胺基酸及其鹽類。

[0013]根據本發明，當形成6-ACA及視需要可形成已內醯胺時並未發現任何導致產率損耗之中間產物的非期望的環化。

[0014]預期本發明方法允許比較WO 2005/68643所述方法獲得可相媲美的或甚至更佳的產率。預期本發明方法用於利用有機體，特別考慮有機體的生長及維持方法時特別有利。

[0015]進一步預期於本發明之實施例中，可改良本發明方法中之6-ACA的生產力(所形成之克/升·小時)。

[0016]如此處使用「或」一詞除非另行規定否則定義爲「及/或」。

[0017]如此處使用「一」一詞除非另行規定否則定義爲「至少一個」。

[0018]當以單數形述及一個名詞(例如一化合物、一添加劑等)時表示含括複數形。

[0019]當述及存在有立體異構物之化合物時，該化合物可爲此種立體異構物中之任一者或其組合物。如此，當述及例如存在有對映異構物之胺基酸時，該胺基酸可爲L-對映異構物、D-對映異構物或其組合物。於存在有天然立體

異構物之情況下，該化合物較佳爲天然立體異構物。

[0020]當述及一種酶而括出酶類別(EC)時，該酶類別爲酶係基於或可基於由「國際生物化學及分子生物學協會命名委員會」(NC-IUBMB)所提供之「酶命名」歸類的類別，該命名可參考http://www.chem.qmul.ac.uk/iubmb/enzyme/。意圖含括未曾(尚未)歸類於特定類別但可以此種方式歸類之其它適當酶。

[0021]「同系物」一詞用於此處特別係指具有至少30%，較佳至少40%，更佳至少60%，更佳至少65%，更佳至少70%，更佳至少75%，更佳至少80%，特佳至少85%，更特佳至少90%，至少91%，至少92%，至少93%，至少94%，至少95%，至少96%，至少97%，至少98%或至少99%序列相同度之多核苷酸或多胜肽。「同系物」一詞也表示包括由於遺傳密碼的簡併性而與另一種核苷酸序列不同但編碼相同多核苷酸序列之核苷酸序列(多核苷酸序列)。

[0022]序列相同度或類似度於此處定義爲經由比較二序列判定兩個或多個多胜肽序列或兩個或多個核苷酸序列間之關係。通常序列相同度或類似度係比較序列的全長，但也可只比較彼此對齊校準的序列部分。業界中，「相同度」或「類似度」也表示由此等序列間之匹配情況判定的多胜肽序列或核苷酸序列(視情況而定)間之序列相關程度。較佳相同度或類似度之測定方法係設計來獲得所試驗之序列間的最大匹配。於本發明之內文中，特定二序列間之相同度與類似度之較佳電腦程式方法包括BLASTP及BLASTN

(Altschul, S. F.等人，J. Mol. Biol. 1990, 215, 403-410，公開得自NCBI及其它來源(BLAST手冊，Altschul, S.等人，NCBI NLM NIH馬里蘭州貝色拉，20894))。較佳使用BLASTP進行多胜肽序列比較之參數爲間隙開口10.0，間隙延長0.5，Blosum 62矩陣。使用BLASTN進行核酸序列比較之較佳參數爲間隙開口10.0，間隙延長0.5，DNA全矩陣(DNA相同度矩陣)。

[0023]根據本發明，使用生物催化劑，換言之該方法之一個反應步驟係藉得自生物來源例如有機體或衍生自有機體之生物分子所衍生之生物材料或生物部分所催化。生物催化劑特別包含一種或多種酶。生物催化劑可以任一種形式使用。於一個實施例中，使用例如呈溶液、乳液、分散液、凍乾細胞(之懸浮液)、溶解產物、或制動於撐體上而分離自天然環境(分離自已經製造該酶之有機體)之一種或多種酶。於一個實施例中，一種或多種酶形成活有機體(例如活全細胞)之一部分。

[0024]酶可於細胞內部進行催化功能。也可能酶可分泌入細胞存在於其中之該介質中。

[0025]活細胞可爲生長中的細胞、休息靜止細胞或休眠細胞(例如孢子或稱芽胞)或處於穩定期的細胞。也可使用已通透化細胞(換言之，變成可通透酶的基質或酶的基質前驅物)之酶形成部分。

[0026]本發明方法所使用之生物催化劑主要爲任一種有機體或可得自或衍生自任何有機體。有機體可爲真核生

物或原核生物。特別有機體可選自於動物(包括人類)、植物、細菌、古菌、酵母及真菌。

[0027]於一個實施例中，生物催化劑係源自於動物，特別源自於動物之一部分例如肝、胰、腦、腎、心或其它器官。動物特別可選自於由哺乳動物所組成的組群，更特別係選自於由兔科(Leporidae)、鼠科(Muridae)、豬科(Suidae)及牛科(Bovidae)所組成之組群。

[0028]適當植物特別包括選自於由鐵角蕨屬(Asplenium)；葫蘆科(Cucurbitaceae)特別爲南瓜屬(Cucurbita)例如Cucurbita moschata(南瓜)、或甜瓜屬(Cucumis)；山靛屬(Mercurialis)例如多年生山靛(Mercurialis perennis)；大風子屬(Hydnocarpus)；及佳樂樹屬(Ceratonia)所組成之組群之植物。

[0029]適當細菌特別係選自於由弧菌屬(Vibrio)、假單胞菌屬(Pseudomonas)、芽胞桿菌屬(Bacillus)、棒桿菌屬(Corynebacterium)、短桿菌屬(Brevibacterium)、腸球菌屬(Enterococcus)、鏈球菌屬(Streptococcus)、克雷白氏菌屬(Klebsiella)、乳球菌屬(Lactococcus)、乳桿菌屬(Lactobacillus)、梭菌屬(Clostridium)、埃希氏菌屬(Escherichia)、棲熱菌屬(Thermus)、分枝桿菌屬(Mycobacterium)、發酵單胞菌屬(Zymomonas)、變形桿菌屬(Proteus)、土壤桿菌屬(Agrobacterium)、地桿菌屬(Geobacillus)、不動桿菌屬(Acinetobacter)、拉斯東氏菌屬(Ralstonia)、紅細菌屬(Rhodobacter)、副球菌屬

(Paracoccus)、新鞘菌屬(Novosphingobium)、亞硝酸菌屬(Nitrosomonas)、退伍軍人症桿菌屬(Legionella)、奈瑟氏菌屬(Neisseria)、紅假單胞菌屬(Rhodopseudomonas)、葡萄球菌屬(Staphylococcus)、迪諾氏球菌屬(Deinococcus)及沙門氏菌屬(Salmonella)所組成之組群。

[0030]適當古菌特別可選自於由古球菌屬(Archaeoglobus)、需氧古菌屬(Aeropyrum)、親鹽桿菌屬(Halobacterium)、甲烷八聯球菌屬(Methanosarcina)、甲烷球菌屬(Methanococcus)、熱原體屬(Thermoplasma)、熱桿菌屬(Pyrobaculum)、甲烷暖球菌屬(Methanocaldococcus)、甲烷桿菌屬(Methanobacterium)、甲烷球菌屬(Methanosphaera)、甲烷古菌屬(Methanopyrus)及甲烷短桿菌屬(Methanobrevibacter)所組成之組群。

[0031]適當真菌特別係選自於由根黴屬(Rhizopus)、紅黴屬(Neurospora)、青黴屬(Penicillium)及麴菌屬(Aspergillus)所組成之組群。

[0032]適當酵母特別係選自於由假絲酵母屬(Candida)、漢遜酵母屬(Hansenula)、克魯維酵母屬(Kluyveromyces)及酵母屬(Saccharomyces)所組成之組群。

[0033]熟諳技藝人士顯然可利用具有適當活性之天然生物催化劑(野生型)或天然生物催化劑之突變株於根據本發明之方法。天然生物催化劑之性質可藉熟諳技藝人士已知之生物技術例如分子演化或理論設計而改良。野生型生物催化劑之突變株之製備方式例如可經由使用熟諳技藝人

士已知之突變發生技術(隨機突變發生、位置導向突變發生、導向演化、基因重組等)修改可作爲生物催化劑或可製造生物催化部分(諸如酶)之有機體之編碼DNA而製備。特定言之，DNA可經修改讓DNA編碼與野生型酶差異至少一個胺基酸之酶，因此可編碼比較野生型包含一個或多個胺基酸取代、刪除及/或插入之酶，或突變株組合兩種或多種親代酶序列，或經由影響如此經修改之DNA於適當(宿主)細胞的表現。後者可藉熟諳技藝人士已知方法達成，諸如密碼子最適化方法或密碼子對最適化方法，例如基於WO 2008/000632所述方法。

[0034]突變株生物催化劑就如下一種或多種面相而言可具有改良性質：對酶基質之選擇性、活性、安定性、溶劑耐受性、pH輪廓資料、溫度輪廓資料、酶基質輪廓資料、對抑制之易感性、輔因子利用性及酶基質親和力。經由應用基於熟諳技藝人士已知方法之例如適當高產出量篩選方法或選擇方法可識別具有改良性質之突變株。

[0035]當述及得自特定來源之生物催化劑特別爲酶時，特別表示源自於第一有機體但實際上係於(經遺傳改性之)第二有機體製造之重組生物催化劑特別爲酶含括作爲得自該第一有機體之生物催化劑特別爲酶。

[0036]於本發明之較佳方法中，製備包含於可催化α-酮酸或胺基酸(亦即包含至少一個羧酸基及至少一個胺基之化合物)之去羧化反應之生物催化劑存在下之生物催化(通常爲酶催化)反應。具有此種催化活性之酶因而分別被稱

爲α-酮酸去羧酶或胺基酸去羧酶。

[0037]該酸較佳爲二元酸，其中該生物催化劑對酮基或胺基旁的酸基具有選擇性。

[0038]大致上，適當去羧酶具有α-酮庚二酸去羧酶活性，可催化AKP轉成5-FVA；或具有α-胺庚二酸去羧酶活性，可催化AAP轉成6-ACA。

[0039]可將α-酮酸或胺基酸去羧化之酶特別係選自於去羧酶組群(EC 4.1.1)，較佳係選自於由草醯乙酸去羧酶(EC 4.1.1.3)、二胺庚二酸去羧酶(EC 4.1.1.20)、分支鏈α-酮酸去羧酶(EC 4.1.1.72)、α-酮異戊酸去羧酶、α-酮戊二酸去羧酶(EC 4.1.1.71)、及丙酮酸去羧酶(EC 4.1.1.1)所組成之組群。

[0040]一種或多種其它適當之去羧酶可選自於由草酸去羧酶(EC 4.1.1.2)、乙醯乙酸去羧酶(EC 4.1.1.4)、纈胺酸去羧酶/白胺酸去羧酶(EC 4.1.1.14)、麩胺酸去羧酶(EC 4.1.1.15)、天冬酸1-去羧酶(EC 4.1.1.11)、3-羥麩胺酸去羧酶(EC 4.1.1.16)、鳥胺酸去羧酶(EC 4.1.1.17)、離胺酸去羧酶(EC 4.1.1.18)、精胺酸去羧酶(EC 4.1.1.19)、2-酮戊二酸去羧酶(EC 4.1.1.71)、及二胺丁酸去羧酶(EC 4.1.1.86)所組成之組群。

[0041]去羧酶特別可爲有機體之去羧酶，該有機體係選自於由南瓜；胡瓜；酵母；真菌例如釀酒酵母(Saccharomyces cerevisiae)、喇叭狀假絲酵母(Candida flareri)、漢遜酵母種屬(Hansenula sp.)、馬克斯克魯維酵母(Kluyveromyces

marxianus)、爪哇根黴(Rhizopus javanicus)、及粗糙紅黴(Neurospora crassa)；哺乳動物特別係得自哺乳動物腦及細菌諸如大腸桿菌(Escherichia coli)、乳酸乳球菌(Lactococcus lactis)、結核分枝桿菌(Mycobacterium tuberculosis)、假單胞菌種屬(Pseudomonas sp.)及活動發酵單胞菌(Zymomonas mobilis)所組成之組群。

[0042]丙酮酸去羧酶可源自於釀酒酵母或活動發酵單胞菌。特別可使用得自活動發酵單胞菌之丙酮酸去羧酶突變株I472A。

[0043]可使用得自大腸桿菌(E. coli)之麩胺酸去羧酶、二胺庚二酸去羧酶或天冬酸去羧酶。

[0044]可使用得自粗糙紅黴、麻風桿菌(Mycobacterium leprae)、產氣梭菌(Clostridium perfringens)、短乳桿菌(Lactobacillus brevis)、結核分枝桿菌、鏈球菌或乳球菌之麩胺酸去羧酶。可獲得麩胺酸去羧酶之乳球菌種屬之實例特別包括乳酸乳球菌諸如乳酸乳球菌種系B1157、乳酸乳球菌IFPL730，更特別爲乳酸乳球菌變種麥芽基因(maltigenes)(前名乳酸鏈球菌變種麥芽基因(Streptococcus lactis var. maltigenes))。

[0045]特別可使用得自假單胞菌之草醯乙酸去羧酶。

[0046]可使用得自乳酸乳球菌之分支鏈α-酮酸去羧酶。更特別可使用得自乳酸乳球菌之α-酮異戊酸去羧酶。

[0047]特別可使用得自結核分枝桿菌之α-酮戊二酸去羧酶。

[0048]於本發明之較佳方法中6-ACA之製備包含於胺基施體存在下可催化轉胺反應且選自於轉胺酶(E.C. 2.6.1)之組群之酶存在下進行酶催化反應。

[0049]大致上，適當轉胺酶具有6-胺己酸6-轉胺酶活性，可催化5-FVA之轉成6-ACA；或具有α-胺庚二酸2-轉胺酶活性，可催化AKP轉成AAP。

[0050]轉胺酶特別係選自於由β-胺異丁酸:α-酮戊二酸轉胺酶、β-丙胺酸轉胺酶、天冬酸轉胺酶、4-胺丁酸轉胺酶(EC 2.6.1.19)、L-離胺酸6-轉胺酶(EC 2.6.1.36)、2-胺己二酸轉胺酶(EC 2.6.1.39)、5-胺戊酸轉胺酶(EC 2.6.1.48)、2-胺己酸轉胺酶(EC 2.6.1.67)及離胺酸:丙酮酸6-轉胺酶(EC 2.6.1.71)所組成之組群。

[0051]於一個實施例中，轉胺酶可選自於由丙胺酸轉胺酶(EC 2.6.1.2)、白胺酸轉胺酶(EC 2.6.1.6)、丙胺酸-酮酸轉胺酶(EC 2.6.1.12)、β-丙胺酸-丙酮酸轉胺酶(EC 2.6.1.18)、(S)-3-胺-2-甲基丙酸轉胺酶(EC 2.6.1.22)、L,L-二胺庚二酸轉胺酶(EC 2.6.1.83)所組成之組群。

[0052]轉胺酶特別係選自於得自哺乳動物；山靛屬特別爲多年生山靛，更特別爲多年生山靛之嫩枝；鐵角蕨屬更特別爲單側鐵角蕨(Asplenium unilaterale)或北方鐵角蕨(Asplenium septentrionale)；佳樂樹屬更特別爲長莢佳樂樹(Ceratonia siliqua)；紅細菌屬特別爲球狀紅細菌(Rhodobacter sphaeroides)；葡萄球菌屬特別爲金黃葡萄球菌(Staphylococcus aureus)；弧菌屬特別爲河流弧菌(Vibrio

fluvialis)；假單胞菌屬特別爲綠膿桿菌(Pseudomonas aeruginosa)；紅假單胞菌屬；芽胞桿菌屬特別爲韋氏芽胞桿菌(Bacillus weihenstephanensis)及枯草桿菌(Bacillus subtilis)；退伍軍人症桿菌屬；亞硝酸菌屬；奈瑟氏菌屬；或酵母特別爲釀酒酵母之轉胺酶。

[0053]於酶屬於哺乳動物之酶時，可特別係源自於哺乳動物腎、哺乳動物肝、哺乳動物心或哺乳動物腦。例如適當酶可選自於得自哺乳動物腎之β-胺異丁酸:α-酮戊二酸轉胺酶特別爲得自豬腎之β-胺異丁酸:α-酮戊二酸轉胺酶；得自哺乳動物肝之β-丙胺酸轉胺酶，特別爲得自兔肝之β-丙胺酸轉胺酶；得自哺乳動物心之天冬酸轉胺酶，特別爲得自豬心之天冬酸轉胺酶；得自哺乳動物肝之4-胺丁酸轉胺酶，特別爲得自豬肝之4-胺丁酸轉胺酶；得自哺乳動物腦之4-胺丁酸轉胺酶，特別爲得自人、豬或兔腦之4-胺丁酸轉胺酶；得自紅黴屬之α-酮己二酸-麩胺酸轉胺酶特別爲得自粗糙紅黴之α-酮己二酸-麩胺酸轉胺酶；得自大腸桿菌之4-胺丁酸轉胺酶，或得自棲熱菌屬之α-胺己二酸轉胺酶，特別爲得自嗜熱棲熱菌(Thermus thermophilus)之α-胺己二酸轉胺酶，及得自梭菌之5-胺戊酸轉胺酶特別爲得自胺戊酸梭菌(Clostridium aminovalericum)之5-胺戊酸轉胺酶所組成之組群。適當2-胺己二酸轉胺酶例如也可由島熱桿菌(Pyrobaculum islandicum)提供。

[0054]特定言之，胺基施體可選自於氨、銨離子、胺及胺基酸所組成之組群。適當胺爲第一胺及第二胺。胺基酸

可具有D-組態或L-組態。胺基施體之實例爲丙胺酸、麩胺酸、異丙基胺、2-胺丁烷、2-胺庚烷、苯甲胺、1-苯基-1-胺乙烷、麩胺、酪胺酸、苯基丙胺酸、天冬酸、β-胺異丁酸、β-丙胺酸、4-胺丁酸、及α-胺己二酸。

[0055]於更佳實施例中，用於製備6-ACA之方法包含於氨源之存在下可催化還原胺化反應之酶選自於作用於施體之CH-NH_2基之氧化還原酶(EC 1.4)之組群，特別爲選自於胺基酸去氫酶(E.C. 1.4.1)之組群之酶存在下進行生物催化反應。大致上，適當胺基酸去氫酶具有6-胺基己酸6-去氫酶活性，可催化5-FVA轉成6-ACA；或具有α-胺庚二酸2-去氫酶活性，可催化AKP轉成AAP。特定言之，適當胺基酸去氫酶可選自於由二胺庚二酸去氫酶(EC 1.4.1.16)、離胺酸6-去氫酶(EC 1.4.1.18)、麩胺酸去氫酶(EC 1.4.1.3；EC 1.4.1.4)、及白胺酸去氫酶(EC 1.4.1.9)所組成之組群。

[0056]於一個實施例中，胺基酸去氫酶可選自於被歸類爲以NAD或NADP作爲受體發揮作用之麩胺酸去氫酶(EC 1.4.1.3)、以NADP作爲受體發揮作用之麩胺酸去氫酶(EC 1.4.1.4)、白胺酸去氫酶(EC 1.4.1.9)、二胺庚二酸去氫酶(EC 1.4.1.16)、及離胺酸6-去氫酶(EC 1.4.1.18)之胺基酸去氫酶。

[0057]胺基酸去氫酶特別係源自於有機體，該有機體係選自於由下列所組成之組群：棒桿菌屬特別爲麩胺酸棒桿菌(Corynebacterium glutamicum)；變形桿菌屬特別爲普通變形桿菌(Proteus vulgaris)；土壤桿菌屬特別爲根瘤土壤桿菌(Agrobacterium tumefaciens)；地桿菌屬特別爲脂嗜熱地

桿菌(Geobacillus stearothermophilus)；不動桿菌屬特別爲不動桿菌種屬ADP1；拉斯東氏菌屬特別爲茄形拉斯東氏菌(Ralstonia solanacearum)；沙門氏菌屬特別爲傷寒桿菌(Salmonella typhimurium)；酵母屬特別爲釀酒酵母；短桿菌屬特別爲黃短桿菌(Brevibacterium flavum)；及芽胞桿菌屬特別爲球狀芽胞桿菌(Bacillus sphaericus)、仙人掌芽胞桿菌(Bacillus cereus)及枯草桿菌(Bacillus subtilis)。例如適當胺基酸去氫酶可選自於得自芽胞桿菌屬特別爲球狀芽胞桿菌之二胺庚二酸去氫酶；得自短桿菌種屬之二胺庚二酸去氫酶；得自棒桿菌屬之二胺庚二酸去氫酶，特別爲得自麩胺酸棒桿菌之二胺庚二酸去氫酶；得自變形桿菌屬之二胺庚二酸去氫酶，特別爲得自普通變形桿菌之二胺庚二酸去氫酶；得自土壤桿菌屬特別爲根瘤土壤桿菌之離胺酸6-去氫酶；得自地桿菌屬特別爲得自脂嗜熱地桿菌之離胺酸6-去氫酶；得自不動桿菌屬之以NADH或NADPH作爲輔因子發揮作用之麩胺酸去氫酶(EC 1.4.1.3)，特別爲得自不動桿菌種屬ADP1之麩胺酸去氫酶；得自拉斯東氏菌屬之麩胺酸去氫酶(EC 1.4.1.3)，特別爲得自茄形拉斯東氏菌之麩胺酸去氫酶；得自沙門氏菌屬之以NADPH作爲輔因子發揮作用之麩胺酸去氫酶(EC 1.4.1.4)，特別爲得自傷寒桿菌之麩胺酸去氫酶；得自酵母屬之麩胺酸去氫酶(EC 1.4.1.4)，特別爲得自釀酒酵母之麩胺酸去氫酶；得自短桿菌屬之麩胺酸去氫酶(EC 1.4.1.4)，特別爲得自黃短桿菌之麩胺酸去氫酶；及得自芽胞桿菌屬之白胺酸去氫酶，特別爲得自仙人

掌芽胞桿菌或枯草桿菌之白胺酸去氫酶。

[0058]於一個特定實施例中，於去羧酶或其它可催化此種轉化之生物催化劑存在下，AKP被生物催化轉化成爲5-甲醯戊酸(5-FVA)。根據本發明使用之去羧酶特別係選自於由下列所組成之組群：得自乳酸乳桿菌、乳酸乳桿菌變種麥芽基因或乳酸乳桿菌亞種石狀(Lactococcus lactis subsp. cremoris)之α-酮酸去羧酶；得自乳酸乳桿菌種系B1157或乳酸乳桿菌IFPL730之分支鏈α-酮酸去羧酶；得自釀酒酵母、喇叭狀假絲酵母、活動發酵單胞菌、漢遜酵母種屬、爪哇根黴、粗糙紅黴、或馬克斯克魯維酵母之丙酮酸去羧酶；得自結核分枝桿菌之α-酮戊二酸去羧酶；得自大腸桿菌、短乳酸桿菌、麻風桿菌、粗糙紅黴或綠膿桿菌之麩胺酸去羧酶；及得自大腸桿菌之天冬酸去羧酶。

[0059]特別發現得自大腸桿菌、活動發酵單胞菌、釀酒酵母、結核分枝桿菌、假單胞菌種屬或乳酸乳桿菌之去羧酶適合用於催化AKP之轉成5-FVA。更特定言之，可使用具有以序列ID 31、序列ID 34、序列ID 37、序列ID 40、序列ID 43、序列ID 46或其同系物識別之胺基酸序列之去羧酶的生物催化劑。也預期此種去羧酶可用於由AAP製備6-ACA。

[0060]隨後5-FVA轉成6-ACA。可以化學方式進行：經由使用氫化催化劑例如鎳於SiO_2/Al_2O_3撐體，藉5-FVA與氨之還原胺化反應，可以高產率製備6-ACA，如EP-A 628 535或DE 4 322 065對9-胺壬酸(9-胺天竺葵酸)及12-胺十二烷酸(12-胺月桂酸)所述。另外，經由使用藉5-FVA與羥胺反應

所製備之6-肟己酸，使用PtO_2氫化6-肟己酸可製備6-ACA (例如參考F.O. Ayorinde, E.Y. Nana, P.D. Nicely, A.S. Woods, E.O. Price, C.P. Nwaonicha J. Am. Oil Chem. Soc. 1997, 74, 531-538有關同系12-胺十二烷酸之合成。

[0061]於一個實施例中，5-FVA轉成6-ACA之轉化係於(i)胺基施體及(ii)轉胺酶、胺基酸去氫酶或其它可催化此種轉化之生物催化劑存在下以生物催化方式進行。特定言之，於此種實施例中，轉胺酶可選自於由下列所組成之組群：得自河流弧菌、綠膿桿菌、枯草桿菌、韋氏芽胞桿菌或大腸桿菌之轉胺酶；得自豬腎之β-胺異丁酸：α-酮戊二酸轉胺酶；得自兔肝之β-丙胺酸轉胺酶；得自多年生山靛嫩枝之轉胺酶；得自豬肝或得自人、兔或豬腦之4-胺丁酸轉胺酶；得自兔肝之β-丙胺酸轉胺酶；及L-離胺酸：α-酮戊二酸-ε-轉胺酶。於使用胺基酸去氫酶之情況下，此種胺基酸去氫酶特別係選自於由得自根瘤土壤桿菌或脂嗜熱地桿菌之離胺酸6-去氫酶所組成之組群。其它適當胺基酸去氫酶可選自於由得自球狀芽胞桿菌、短桿菌種屬、麩胺酸棒桿菌、或普通變形桿菌之二胺庚二酸去氫酶所組成之組群；由得自不動桿菌種屬ADP1或茄形拉斯東氏菌之以NADH或NADPH作爲輔因子發揮作用之麩胺酸去氫酶(EC 1.4.1.3)所組成之組群；由得自傷寒桿菌之以NADPH作爲輔因子作用之麩胺酸去氫酶(EC 1.4.1.4)所組成之組群；由得自釀酒酵母或黃短桿菌之麩胺酸去氫酶(EC 1.4.1.4)所組成之組群；或由得自仙人掌芽胞桿菌或枯草桿菌之白胺酸去

氫酶所組成之組群。

[0062]於特定實施例中，藉包含以序列ID 2、序列ID 5、序列ID 8、序列ID 65、序列ID 67、序列ID 69或任何此等序列之同系物識別之胺基酸序列之轉胺酶的生物催化劑催化5-FVA轉成6-ACA。

[0063]於特定實施例中，AKP以化學方式轉成5-FVA。經由於共沸水移除且同時喪失二氧化碳下，使得第二胺例如味啉進行中間烯胺之形成，可進行2-酮羧酸有效被化學去羧化成爲相對應之醛，該方法例如係基於四面體函件1982,23(4), 459-462所述之方法。中間物端基烯醯胺隨後被水解成爲相對應之醛。經由於轉胺酶存在下進行轉胺化反應，或經由藉胺基酸去氫酶或其它可催化此種轉化的生物催化劑以酶還原胺化，5-FVA隨後以生物催化方式轉成6-ACA。此種轉胺酶或胺基酸去氫酶特別可選自於前文當說明5-FVA轉成6-ACA時所述之生物催化劑。

[0064]另外，5-FVA轉成6-ACA可藉化學方法例如前文說明方法進行。

[0065]於特定實施例中，於(i)轉胺酶、胺基酸去氫酶、或其它可催化此種轉化之生物催化劑及(ii)胺基施體存在下，AKP被生物催化地轉成AAP。此種根據本發明用於將AKP轉成AAP之轉胺酶特別係選自於前文說明之轉胺酶更特別係選自於由下列所組成之組群：得自豬心之天冬酸轉胺酶；得自粗糙紅黴或酵母之α-酮己二酸：麩胺酸轉胺酶；得自多年生山靛嫩枝之轉胺酶；得自大腸桿菌之4-胺丁酸

轉胺酶；得自嗜熱棲熱菌之α-胺己二酸轉胺酶；得自北方鐵角蕨或單側鐵角蕨之轉胺酶；及得自長莢佳樂樹之轉胺酶。

[0066]於較佳實施例中，用於將AKP轉成AAP之轉胺酶係選自於由得自弧菌屬、假單胞菌屬、芽胞桿菌屬、退伍軍人症桿菌屬、亞硝酸菌屬、奈瑟氏菌屬、紅桿菌屬、埃希氏菌屬及紅假單胞菌屬之轉胺酶所組成之組群。

[0067]特別發現得自選自於由下列所組成之組群之有機體之轉胺酶適合用於催化將AKP轉成AAP：枯草桿菌、球狀紅桿菌、嗜肺性退伍軍人症桿菌、歐洲亞硝酸菌、淋病奈瑟氏菌、環狀假單胞菌(Pseudomonas syringae)、沼澤紅假單胞菌(Rhodopseudomonas palustris)、河流弧菌、大腸桿菌及綠膿桿菌。

[0068]於特定實施例中，爲了將AKP轉成AAP，使用包含根據序列ID 2、序列ID 8、序列ID 12、序列ID 15、序列ID 17、序列ID 19、序列ID 21、序列ID 23、序列ID 25、序列ID 27、序列ID 29或此等序列中之任一者之同系物之胺基酸序列之轉胺酶。

[0069]於又一個實施例中，用於製備AAP之方法包含於氨源之存在下可催化還原胺化反應之酶選自於作用於施體之CH-NH_2基之氧化還原酶(EC 1.4)之組群，特別爲選自於胺基酸去氫酶(E.C. 1.4.1)之組群之酶存在下進行生物催化反應。大致上，適當胺基酸去氫酶具有α-胺庚二酸2-去氫酶活性，可催化AKP轉成AAP。

[0070]特別適當胺基酸去氫酶可選自於由二胺庚二酸去氫酶(EC 1.4.1.16)、麩胺酸去氫酶(EC 1.4.1.3;EC 1.4.1.4)及白胺酸去氫酶(EC 1.4.1.9)所組成之組群。

[0071]於一個實施例中，胺基酸去氫酶係選自於歸類爲以NAD及NADP作爲受體發揮作用之麩胺酸去氫酶(EC 1.4.1.3)、以NADP作爲受體發揮作用之麩胺酸去氫酶(EC 1.4.1.4)、白胺酸去氫酶(EC 1.4.1.9)、及二胺庚二酸去氫酶(EC 1.4.1.16)之胺基酸去氫酶。

[0072]胺基酸去氫酶特別係源自於選自於由下列所組成之組群之有機體：棒桿菌屬特別爲麩胺酸棒桿菌；變形桿菌屬特別爲普通變形桿菌；土壤桿菌屬特別爲根瘤土壤桿菌；地桿菌屬特別爲脂嗜熱地桿菌；不動桿菌屬特別爲不動桿菌種屬ADP1；拉斯東氏菌屬特別爲茄形拉斯東氏菌；沙門氏菌屬特別爲傷寒桿菌；酵母屬特別爲釀酒酵母；短桿菌屬特別爲黃短桿菌；及桿菌屬特別爲球狀芽胞桿菌、仙人掌芽胞桿菌(Bacillus cereus)及枯草桿菌。

[0073]例如適當胺基酸去氫酶可選自於得自芽胞桿菌屬特別爲球狀芽胞桿菌之二胺庚二酸去氫酶；得自短桿菌種屬之二胺庚二酸去氫酶；得自棒桿菌屬之二胺庚二酸去氫酶，特別爲得自麩胺酸棒桿菌之二胺庚二酸去氫酶；得自變形桿菌屬之二胺庚二酸去氫酶，特別爲得自普通變形桿菌之二胺庚二酸去氫酶；得自不動桿菌屬之以NADH或NADPH作爲輔因子發揮作用之麩胺酸去氫酶(EC 1.4.1.3)，特別爲得自不動桿菌種屬ADP1之麩胺酸去氫酶；

得自拉斯東氏菌屬之麩胺酸去氫酶(EC 1.4.1.3)，特別爲得自茄形拉斯東氏菌之麩胺酸去氫酶；得自沙門氏菌屬以NADPH作爲輔因子發揮作用之麩胺酸去氫酶(EC 1.4.1.4)，特別爲得自傷寒桿菌之麩胺酸去氫酶；得自酵母屬之麩胺酸去氫酶(EC 1.4.1.4)，特別爲得自釀酒酵母之麩胺酸去氫酶；得自短桿菌屬之麩胺酸去氫酶(EC 1.4.1.4)，特別爲得自黃短桿菌之麩胺酸去氫酶；及得自芽胞桿菌屬之白胺酸去氫酶，特別爲得自仙人掌芽胞桿菌或枯草桿菌之白胺酸去氫酶。

[0074]另一種適當胺基酸去氫酶可選自於由得自根瘤土壤桿菌或脂嗜熱地桿菌之離胺酸6-去氫酶所組成之組群；或由得自仙人掌芽胞桿菌或枯草桿菌之白胺酸去氫酶所組成之組群。

[0075]於本發明方法所製備之AAP進一步可用於6-ACA之製備。發明人已經實現由AKP所製成之AAP可藉去羧化反應而轉成6-ACA。此反應可以化學方式進行，例如經由於酮或醛催化劑存在下於高沸溶劑中加熱進行。例如，胺基酸係以良好產率於環己醇於150-160℃使用1-2 v/v%環己烯酮去羧化，如M.Hashimoto, Y. Eda, Y. Osanai, T. Iwai及S. Aoki於Chem. Lett. 1986, 893-896所述。類似方法係說明於Daiso之Eur. Pat. Appl. 1586553，及S.D. Brandt, D. Mansell, S. Freeman, I.A. Fleet, J.F. Alder J. Pharm. Biomed. Anal. 2006, 41, 872-882。

[0076]另外，AAP去羧化成爲6-ACA可於去羧酶或其它

催化此種去羧化之生物催化劑存在下以生物催化方式進行。

[0077]去羧酶可選自於可催化α-胺基酸之去羧化之去羧酶。可催化α-胺基酸之酶特別可選自於由去羧酶(E.C. 4.1.1)之組群，較佳係選自於由丙酮酸去羧酶(EC 4.1.1.1)、二胺庚二酸去羧酶(EC 4.1.1.20)、二胺庚二酸去羧酶(EC 4.1.1.20)、分支鏈α-酮酸去羧酶(EC 4.1.1.72)其包括α-酮異戊酸去羧酶及α-酮戊二酸去羧酶(EC 4.1.1.71)所組成之組群。

[0078]一種或多種其它適當去羧酶特別可選自於由下列所組成之組群：草酸去羧酶(EC 4.1.1.2)、草醯乙酸去羧酶(EC 4.1.1.3)、乙醯乙酸去羧酶(EC 4.1.1.4)、天冬酸1-去羧酶(EC 4.1.1.11)、纈胺酸去羧酶/白胺酸去羧酶(EC 4.1.1.14)、麩胺酸去羧酶(EC 4.1.1.15)、3-羥麩胺酸去羧酶(EC 4.1.1.16)、鳥胺酸去羧酶(EC 4.1.1.17)、離胺酸去羧酶(EC 4.1.1.18)、精胺酸去羧酶(EC 4.1.1.19)、2-酮戊二酸去羧酶(EC 4.1.1.71)、及二胺丁酸去羧酶(EC 4.1.1.86)。

[0079]去羧酶特別爲選自於下列所組成之組群之有機體之去羧酶：南瓜，例如南瓜；胡瓜；酵母；真菌例如釀酒酵母、喇叭狀假絲酵母、漢遜酵母種屬、馬克斯克魯維酵母、爪哇根黴、及粗糙紅黴；哺乳動物特別係得自哺乳動物腦及細菌諸如大腸桿菌、乳酸乳球菌、結核分枝桿菌、假單胞菌種屬及活動發酵單胞菌所組成之組群。

[0080]丙酮酸去羧酶可源自於釀酒酵母或活動發酵單

胞菌。特別可使用得自活動發酵單胞菌之丙酮酸去羧酶突變株I472A。特別可使用得自假單胞菌之草醯乙酸去羧酶。可使用得自大腸桿菌(E. coli)之麩胺酸去羧酶、二胺庚二酸去羧酶或天冬酸去羧酶，或得自粗糙紅黴、麻風桿菌、產氣梭菌、短乳桿菌、結核分枝桿菌、鏈球菌或乳球菌之麩胺酸去羧酶。可獲得麩胺酸去羧酶之乳球菌種屬之實例特別包括乳酸乳球菌諸如乳酸乳球菌種系B1157、乳酸乳球菌IFPL730，更特別爲乳酸乳球菌變種麥芽基因(前名乳酸鏈球菌變種麥芽基因)。二胺庚二酸去羧酶例如可得自可由二胺庚二酸合成離胺酸之有機體。此種有機體特別係得自細菌、古菌及植物。特別二胺庚二酸去羧酶可得自革蘭氏陰性菌例如大腸桿菌。可使用得自乳酸乳桿菌之分支鏈α-酮酸去羧酶。更特別，可使用得自乳酸乳桿菌之分支鏈α-酮酸去羧酶及α-酮異戊酸去羧酶。

[0081]特別可使用得自結核分枝桿菌之α-酮戊二酸去羧酶。發明人發現得自結核分枝桿菌之α-酮戊二酸去羧酶(Kgd)可用於將AAP轉成6-ACA。特別，發明人發現包含如序列ID 46號所示序列或其功能類似物之此種去羧酶可催化由AAP形成6-ACA。

[0082]麩胺去羧酶特別係選自於南瓜、胡瓜、酵母、或小牛腦；及二胺庚二酸去羧酶(EC 4.1.1.20)。

[0083]二胺庚二酸去羧酶例如可得自可由二胺庚二酸合成離胺酸之有機體。此種有機體特別係得自細菌、古菌及植物。

[0084]特別二胺庚二酸去羧酶可得自革蘭氏陰性菌例如大腸桿菌。

[0085]於特定實施例中，AKP藉化學方式轉成AAP。如對類似化合物所述，藉催化魯卡特-瓦拉(Leuckart-Wallach)反應，可由2-酮庚二酸製備AAP。本反應係使用甲酸銨於甲醇及$[RhCp^*Cl_2]_2$作爲均質催化劑進行(M. Kitamura, D. Lee, S. Hayashi, S. Tanaka, M. Yoshimura J. Org. Chem. 2002, 67, 8685-8687)。另外，魯卡特-瓦拉反應可以水性甲酸銨使用$[Ir^{III}Cp^*(bpy)H_2O]SO_4$作爲催化劑，如S. Ogo, K. Uehara及S. Fukuzumi於J. Am. Chem. Soc. 2004, 126, 3020-3021所述進行。α-酮酸轉換成爲(對映異構物豐富的)胺基酸藉由與(對掌性)苄基胺反應以及隨後以Pd/C或$Pd(OH)_2$/C氫化中間物亞胺也可能達成。例如參考R.G. Hiskey, R.C. Northrop J. Am. Chem. Soc. 1961, 83, 4798。

[0086]隨後於去羧酶或其它可進行此種去羧化反應之生物催化劑存在下，AAP以生物催化方式轉成6-ACA。此種去羧酶特別可選自於前文當描述用於將AAP轉成6-ACA之生物催化劑時所述之該等生物催化劑。

[0087]另外，AAP轉成6-ACA之轉化可藉化學方法例如前述方法進行。

[0088]於特定實施例中，於去羧酶或可催化此種轉化之其它生物催化劑存在下，AKP以生物催化方式轉成5-FVA；及隨後於轉胺酶、胺基酸去氫酶、或其它可催化此種轉化之生物催化劑存在下，5-FVA轉成6-ACA。適合用於此種反

應之去羧酶特別係選自於前文描述AKP生物催化成爲5-FVA時所述之去羧酶所組成之組群。用於轉化5-FVA之適當轉胺酶或胺基酸去氫酶特別可選自於前文於描述5-FVA生物催化轉化成爲6-ACA之生物催化時所述者。

[0089]於特定實施例中，於轉胺酶、胺基酸去氫酶、或其它可催化此種轉化之生物催化劑存在下，AKP被生物催化轉成AAP；及隨後於去羧酶或其它可催化此種轉化之生物催化劑存在下，AAP被轉成6-ACA。

[0090]適合用於此種反應之酶特別係選自於前文當描述AKP轉成AAP之生物轉化及AAP轉成6-ACA之生物轉化時所述之轉胺酶、胺基酸去氫酶及去羧酶所組成之組群。

[0091]用於製備6-ACA之AKP原則上可以任一種方式獲得。例如AKP可基於H. Jäger等人，Chem. Ber. 1959, 92, 2492-2499所述方法獲得。AKP之製備方式可經由使用乙氧化鈉作爲鹼以草酸二乙酯烷化環戊酮，於強酸(2M鹽酸)中回流所得產物及例如藉由甲苯結晶而回收產物，製備AKP。

[0092]也可由天然來源例如由甲烷產生性古菌、由北方鐵角蕨、或由驅蟲大風子(Hydnocarpus anthelminthica)之AKP。AKP例如可萃取自此等有機體或其部分例如驅蟲大風子種子。適當萃取方法例如係基於A.I. Virtanen及A.M. Berg於Acta Chemica Scandinavica 1954, 6, 1085-1086所述方法，其中描述使用70%乙醇而由鐵角蕨萃取胺基酸及AKP。

[0093]於特定實施例中，AKP係於一種方法製備，包含

將α-酮戊二酸(AKG)轉成α-酮己二酸(AKA)及將α-酮己二酸轉成α-酮庚二酸。本反應可藉生物催化劑催化。AKG例如可以技藝界已知方式，由碳源例如碳水化合物而以生物催化方式製備。

[0094]用於由AKG製備AKP之適當催化劑特別可選自於催化α-酮戊二酸之C_1-延長成爲α-酮己二酸及/或α-酮己二酸之C_1-延長成爲α-酮庚二酸之生物催化劑。

[0095]於特定實施例中，AKP之製備係藉生物催化劑催化，該生物催化劑包含

a. AksA酶或其同系物；

b. 選自於由AksD酶、AksE酶、AksD酶同系物及AksE酶同系物所組成之組群之至少一種酶；及

c. AksF酶或其同系物。

[0096]AksA, AksD, AksE, AksF酶或其同系物中之一者或多者可得自選自於甲烷產生性古菌所組成之組群之有機體，較佳係選自於由甲烷球菌、甲烷暖球菌、甲烷八聯球菌、甲烷熱桿菌(Methanothermobacter)、甲烷球菌、甲烷古菌及甲烷短桿菌所組成之組群。

[0097]於特定實施例中，催化由α-酮戊二酸(AKG)製備AKP之生物催化劑包含催化α-酮戊二酸轉成α-酮己二酸之轉化之酶系，其中該酶係形成用於離胺酸生物合成之α-胺己二酸路徑之一部分。「酶系」一詞特別於此處用於指其可催化特定轉化的單一種酶或一組酶。

[0098]由AKG製備AKP包含使用已知或未知中間物之

一種或多種生物催化反應例如AKG轉成AKA或AKA轉成AKP。此等系統可存在於細胞內部或可由細胞分離。酶系特別係得自選自於由酵母、真菌、古菌及細菌所組成之組群，特別係得自於由下列所組成之組群：青黴屬、頭孢子菌屬(Cephalosporium)、貝利菌屬(Paelicomyces)、髮癬菌屬(Trichophytum)、麴菌屬、白腐菌屬(Phanerochaete)、翅孢子菌屬(Emericella)、黑穗病菌屬(Ustilago)、裂殖酵母屬(Schizosaccharomyces)、酵母屬、假絲酵母屬、雅羅酵母屬(Yarrowia)、畢赤酵母屬(Pichia)、克魯維酵母屬、棲熱菌屬、迪諾球菌屬、高溫球菌屬(Pyrococcus)、硫葉菌屬(Sulfolobus)、熱球菌屬(Thermococcus)、甲烷球菌屬、甲烷暖球菌屬、甲烷球菌屬、甲烷古菌屬、甲烷短桿菌屬、甲烷八聯球菌屬及甲烷熱桿菌屬。

[0099]於特定實施例中，催化由α-酮戊二酸製備AKP之生物催化劑包含催化α-酮戊二酸轉成α-酮己二酸之轉化之酶系，其中酶系中之酶中之至少一者係源自於選自於由藍藻、根瘤菌、γ-蛋白菌及放線桿菌所組成之組群之固氮菌，特別係選自於由念珠藻屬(Anabaena)、微胞藻屬(Microcystis)、群胞藻屬(Synechocystis)、根瘤菌屬(Rhizobium)、緩根瘤菌屬(Bradyrhizobium)、假單胞菌屬、固氮菌屬(Azotobacter)、克雷白氏菌屬及法蘭克氏菌屬(Frankia)所組成之組群。

[0100]此等Aks酶之同系物及編碼此等酶之基因之實例列舉於以下二頁之表1A及表1B。

表1A

酶 名稱	有機體	基因	蛋白質
AksA	甲烷暖球菌	MJ0503	NP_247479
	甲烷熱桿菌ΔH	MTH1630	NP_276742
	甲烷球菌S2	MMP0153	NP_987273
	甲烷球菌C5	MmarC5_1522	YP_001098033
	甲烷球菌C7	MmarC7_1153	YP_001330370
	甲烷球菌DSM 3091	Msp_0199	YP_447259
	甲烷古菌AV19	MK1209	NP_614492
	甲烷短桿菌ATCC35061	Msm_0722	YP_001273295
	甲烷球菌SB	Mevan_1158	YP_001323668
	甲烷球菌3	Maeo_0994	YP_001325184
AksD	甲烷暖球菌	MJ1003	NP_247997
	甲烷熱桿菌ΔH	MTH1386	NP_276502
	甲烷球菌S2	Mmp1480	NP_988600
	甲烷球菌C5	MmarC5_0098	YP_001096630
	甲烷球菌C7	MmarC7_0724	YP_001329942
	甲烷球菌DSM 3091	Msp_1486	YP_448499
	甲烷古菌AV19	MK1440	NP_614723
	甲烷短桿菌ATCC35061	Msm_0723	YP_001273296
	甲烷球菌SB	Mevan_0789	YP_001323307
	甲烷球菌3	Maeo_0311	YP_001324511

基因及蛋白質可參考www.ncbi.nlm.nih.gov/, (2008年4月15日取得)

表1B

酶 名稱	有機體	基因	蛋白質
AksE	甲烷暖球菌	MJ1271	NP_248267
	甲烷熱桿菌ΔH	MTH1387	NP_276503
	甲烷球菌S2	MMP0381	NP_987501
	甲烷球菌C5	MmarC5_1257	YP_001097769
	甲烷球菌C7	MmarC7_1379	YP_001330593
	甲烷球菌DSM 3091	Msp_1485	YP_448498
	甲烷古菌AV19	MK0781	NP_614065
	甲烷短桿菌ATCC35061	Msm_0847	YP_001273420
	甲烷球菌SB	Mevan_1368	YP_001323877
	甲烷球菌3	Maeo_0652	YP_001324848
AksF	甲烷暖球菌	MJ1596	NP_248605
	甲烷熱桿菌ΔH	MTH184	NP_275327
	甲烷球菌S2	MMP0880	NP988000
	甲烷球菌C5	MmarC5_0688	YP001097214
	甲烷球菌C7	MmarC7_0128	YP_001329349
	甲烷球菌DSM 3091	Msp_0674	YP_447715
	甲烷古菌AV19	MK0782	NP_614066
	甲烷短桿菌ATCC35061	Msm_0373	YP001272946
	甲烷球菌SB	Mevan_0040	YP_001322567
	甲烷球菌3	Maeo_1484	YP_001325672

基因及蛋白質可參考www.ncbi.nlm.nih.gov/, (2008年4月15日取得)

[0101]若有所需，根據本發明所得之6-ACA可經環化而形成己內醯胺，如US-A 6,194,572所述。

[0102]於本發明之內文中用於任何生物催化步驟之反應條件可依據生物催化劑特別爲酶已知條件、此處揭示之資訊及任選地若干例行實驗而選用。

[0103]原則上，所使用之反應介質之pH可選自寬廣限度，只要於pH條件下生物催化劑具有活性即可。依據生物催化劑及其它因素而定，可使用鹼性條件、中性條件及酸性條件。於該方法包括微生物之情況下，例如用於表現可催化本發明方法之酶，pH係選擇讓微生物可進行其期望的功能。於25℃主要爲水性系統之情況下，pH特別可選自於低於中性pH四個pH單位至高於中性pH兩個pH單位之範圍，亦即選自於pH 3至pH 9。若水爲唯一溶劑或主要溶劑(>50wt.%，特別>90wt.%，以總液體爲基準)，則該系統被視爲水性，其中小量醇或其它溶劑(<50wt.%，特別<10wt.%，以總液體爲基準)可以可維持微生物活性存在的濃度而溶解(例如作爲碳源)。特別於使用酵母及/或真菌之情況下，基於25℃大致爲水性系統，以酸性條件爲佳，特別pH於pH 3至pH 8之範圍。若有所需，可使用酸及/或鹼或使用酸與鹼之適當組合緩衝而調整pH。

[0104]原則上，培養條件可於寬廣範圍選用，只要生物催化劑顯示充分活性及/或生長即可。如此包括有氧、微有氧、氧限制及無氧條件。

[0105]無氧條件於此處定義爲不含任何氧氣或實質上

無任何氧被生物催化劑耗用之情況，特別爲微生物，通常係相當於氧氣耗用量低於5毫莫耳/升·小時，特別爲氧耗用量低於2.5毫莫耳/升·小時或低於1毫莫耳/升·小時。

[0106]有氧條件爲其中供無限制生長之夠高氧濃度溶解於介質，可支援至少10毫莫耳/升·小時，更佳大於20毫莫耳/升·小時，又更佳大於50毫莫耳/升·小時，及最佳大於100毫莫耳/升·小時之氧耗用速率之條件。

[0107]氧限制條件定義爲氧耗用量受到氧由氣體轉成液體所限制之條件。氧限制條件之下限係由無氧條件之上限決定，換言之，通常至少爲1毫莫耳/升·小時，且特別至少2.5毫莫耳/升·小時或至少5毫莫耳/升·小時。氧限制條件之上限係由有氧條件之下限決定，亦即低於100毫莫耳/升·小時，低於50毫莫耳/升·小時，低於20毫莫耳/升·小時，或低於10毫莫耳/升·小時。

[0108]條件爲有氧、無氧或氧限制，係依據進行該方法之條件決定，特別係由流入氣流之數量及組成、使用設備之實際混合/質量移轉性質、及所使用之微生物類別及微生物密度決定。

[0109]原則上，所使用之溫度並無特殊限制，只要生物催化劑特別爲酶顯示實質活性即可。大致上溫度至少爲0℃，特別至少爲15℃，更特別至少爲20℃。期望之最高溫度係依據生物催化劑決定。通常此種最高溫度爲技藝界所已知，例如於市售生物催化劑之情況下指示於產品資料單中，或可基於普通常識及此處揭示之資訊而例行性地決

定。溫度通常爲90℃或以下，較佳爲70℃或以下，特別爲50℃或以下，更特別爲40℃或以下。

[0110]特別若生物催化反應係於宿主有機體外側進行，則於使用於此種介質中保有足夠活性之酶時，可使用高濃度(例如大於50 wt.%或大於90 wt.%)之包含有機溶劑之反應介質。

[0111]於較佳方法中，使用6-ACA酶基質之全細胞生物轉換或用於形成6-ACA之中間物(AKP、AAP或5-FVA)製備6-ACA，包含其中製造可催化生物轉換之一種或多種生物催化劑(通常爲一種或多種酶)之微生物，諸如選自於下列所組成之組群之一種或多種生物催化劑：可催化AKP轉成AAP之生物催化劑，可催化AAP轉成6-ACA之生物催化劑，可催化AKP轉成5-FVA之生物催化劑及可催化5-FVA轉成6-ACA之生物催化劑。於較佳實施例中，微生物可製造去羧酶及/或選自於胺基酸去氫酶及轉胺酶中之至少一種酶；可催化前述反應步驟，及用於微生物之碳源。

[0112]碳源特別含有選自於由一元醇、多元醇、羧酸、二氧化碳、脂肪酸、甘油酯，包括包含該等化合物中之任一者之混合物所組成之組群中之至少一種化合物。適當一元醇包括甲醇及乙醇。適當多元醇包括甘油及碳水化合物。適當脂肪酸或甘油酯特別係以食用油形式，較佳爲植物來源形式提供。

[0113]特別可使用碳水化合物，原因在於通常碳水化合物係以大量得自生物可再生來源，諸如農產品較佳爲農業

廢料。較佳使用選自於由葡萄糖、果糖、蔗糖、乳糖、甜菜糖、澱粉、纖維素及半纖維素所組成之組群之碳水化合物。特佳爲葡萄糖、包含葡萄糖之寡醣及包含葡萄糖之多醣。

[0114]包含一種或多種用於本發明方法催化反應步驟之生物催化劑(通常爲一種或多種酶)之細胞，特別爲重組細胞，可使用技藝界已知之分子生物技術組成。例如若於重組細胞(可爲非同質系統)製造一種或多種生物催化劑，則此種技術可用於提供包含編碼一種或多種生物催化劑之一種或多種基因之載體(諸如重組載體)。可使用各自包含一種或多種基因之一種或多種載體。此種載體包含以工作式鏈接至編碼生物催化劑之基因之一個或多個調節元件例如一個或多個啓動基因。

[0115]如此處使用，「工作式鏈接」一詞係指多核苷酸元件(或編碼序列或核酸序列)呈功能關係鏈接。核酸序列當與另一個核酸序列呈功能關係放置時爲「工作式鏈接」。例如啓動基因或加強基因若影響編碼序列之轉錄，則係工作式鏈接至該編碼序列。

[0116]如此處使用，「啓動基因」一詞係指可發揮功能控制一個或多個基因之轉錄之核酸片段，位在相對於基因轉錄起始位置之轉錄方向的上游，且於結構上藉DNA相依性RNA聚合酶之結合位置、轉錄起始位置及任何其它DNA序列的存在加以識別，該等DNA序列包括但非限於轉錄因子結合位置、阻遏基因及活化基因蛋白質結合位置、及熟

諳技藝人士已知可直接或間接作用於調節得自啓動基因之轉錄量之任何其它核苷酸序列。「組成性」啓動基因爲於大部分環境條件及發育條件下具有活性之啓動基因。「誘導性」啓動基因爲於環境調節或發育調節下具有活性之啓動基因。「同源」一詞當用於指示一給定的(重組)核酸或多胜肽分子與一給定的宿主有機體或宿主細胞之關係時，須瞭解表示於自然界該核酸或多胜肽分子係由同種且較佳爲相同變種或相同種系之宿主細胞或宿主有機體所製造。

[0117]可用於達成編碼用於本發明方法之酶特別爲轉胺酶、胺基酸去氫酶或去羧酶之核酸序列之表現的啓動基因，諸如前文說明，可爲編碼欲表現之該酶之核酸序列所本有，或爲其工作式鏈接之核酸序列(編碼序列)之非同源啓動基因。較佳，啓動基因爲宿主細胞之同源性，亦即宿主細胞內生性。

[0118]若使用非同源啓動基因(相對於感興趣之酶之編碼核酸序列爲非同源)，則非同源啓動基因較佳比較該編碼序列原有的啓動基因，可產生更高穩定濃度之包含編碼序列之轉錄本(或每單位時間可產生更多轉錄本分子，亦即mRNA分子)。於本內文中之此種啓動基因包括組成性天然啓動基因及誘導性天然啓動基因以及基因改造啓動基因，爲熟諳技藝人士眾所周知。

[0119]「強力組成性啓動基因」爲造成mRNA以比天然宿主細胞更高頻率起始之啓動基因。於革蘭氏陽性微生物內此種強力組成性啓動基因之實例包括SP01-26、

SP01-15、veg、pyc (丙酮酸羧基酶啓動基因)、及amyE。

[0120]於革蘭氏陽性微生物中之誘導性啓動基因之實例包括IPTG誘導性Pspac啓動基因、木糖誘導性PxylA啓動基因。

[0121]革蘭氏陰性微生物中之組成性啓動基因及誘導性啓動基因之實例包括但非限於tac、tet、trp-tet、lpp、lac、lpp-lac、laclq、T7、T5、T3、gal、trc、ara (P_{BAD})、SP6、λ-P_R、及λ-P_L。

[0122](絲狀)真菌細胞之啓動基因爲技藝界所已知，可爲例如葡萄糖-6-磷酸去氫酶gpdA啓動基因、蛋白酶啓動基因諸如pepA、pepB、pepC、葡萄糖澱粉酶glaA啓動基因、澱粉酶amyA、amyB啓動基因、催化酶catR或catA啓動基因、葡萄糖氧化酶goxC啓動基因、β-半乳糖苷酶lacA啓動基因、α-葡萄糖苷酶aglA啓動基因、轉譯伸長因子tefA啓動基因、木聚糖酶啓動基因諸如xlnA、xlnB、xlnC、xlnD、纖維素酶啓動基因諸如eglA、eglB、cbhA、轉錄調節基因之啓動基因諸如areA、creA、xlnR、pacC、prtT、或其它啓動基因，可參考NCBI網站(http://www.ncbi.nlm.nih.gov/entrez/)。

[0123]就核酸(DNA或RNA)或蛋白質使用時，「同源」一詞係指天然未出現作爲有機體、細胞、基因體或其所存在之DNA或RNA序列之一部分之核酸或蛋白質；或指出現於細胞或出現於與天然出現不同的細胞或基因體位置或DNA或RNA序列之核酸或蛋白質。非同源核酸或蛋白質非

爲該核酸或蛋白質導入其中之該細胞所內生，反而係得自其它細胞或以合成製造或重組製造。大致上但非必要，此種核酸編碼由該DNA所轉錄或表現之細胞非天然製造的蛋白質。類似地外生RNA編碼該外生RNA存在於其中之該細胞非天然表現的蛋白質。非同源核酸及蛋白質也被稱作爲外來核酸或蛋白質。熟諳技藝人士瞭解對表現細胞而言被識別爲非同源或外來的任一種核酸或蛋白質於此處涵蓋於非同源核酸或蛋白質之術語。

[0124]根據本發明之方法可於宿主有機體進行，該宿主有機體可爲新穎。

[0125]如此，本發明亦係關於包含一種或多種生物催化劑之宿主細胞，該生物催化劑可催化本發明方法中之至少一個反應步驟，特別可催化AKP、AAP或5-FVA轉成6-ACA之轉化中之至少一個反應步驟。本發明亦係關於包含編碼一種或多種酶之一種或多種基因之新穎載體，該等酶可催化本發明方法中之至少一個反應步驟，特別可催化AKP轉成6-ACA之轉化中之至少一個反應步驟；且本發明係關於包含編碼一種或多種酶之一種或多種基因之新穎宿主細胞，該酶可催化本發明方法中之至少一個反應步驟，特別可催化AKP轉成6-ACA之轉化中之至少一個反應步驟(該一種或多種基因可構成一種或多種載體之一部分)。

[0126]於一特定實施例中，根據本發明之宿主細胞爲包含編碼一種生物催化劑之核酸序列之重組細胞，該生物催化劑可催化轉胺反應或還原胺化反應來由α-酮庚二酸形成

α-胺庚二酸。此種序列可爲載體之一部分，或可插入染色體DNA。

[0127]特別，根據本發明之宿主細胞或載體包含選自於由下列所組成之組群中之至少一個核酸序列，特別至少兩個核酸序列：編碼具有α-酮庚二酸去羧酶活性之酶之核酸序列、編碼具有5-甲醯戊酸轉胺酶活性之酶之核酸序列、編碼具有α-酮庚二酸轉胺酶活性之酶之核酸序列、編碼具有α-酮庚二酸去氫酶活性之酶之核酸序列、及編碼具有α-胺庚二酸去羧酶活性之酶之核酸序列。此種序列中，典型爲一者或多者特別爲兩者或多者屬於重組序列。

[0128]於較佳實施例中，根據本發明之宿主細胞典型爲重組宿主細胞或載體包含編碼具有α-酮庚二酸去羧酶活性之至少一種生物催化劑之核酸序列，及/或選自於編碼具有5-甲醯戊酸轉胺酶活性之生物催化劑序列中之至少一種核酸序列。

[0129]於此種實施例中，編碼具有α-酮庚二酸去羧酶活性之酶之核酸序列特別包含根據序列ID 31、序列ID 34、序列ID 37、序列ID 40、序列ID 43或序列ID 46或任何此等序列之同系物之胺基酸序列；及/或編碼具有5-甲醯戊酸轉胺酶活性之酶之核酸序列特別包含根據序列ID 2、序列ID 5、序列ID 8、序列ID 65、序列ID 67、序列ID 69或其同系物之胺基酸序列。其中一種或多種核酸序列可構成一種或多種重組載體之一部分。

[0130]於又更佳實施例中，載體或宿主細胞包含編碼具

有α-酮庚二酸轉胺酶活性之核酸序列及/或編碼具有α-胺庚二酸去羧酶活性之核酸序列。編碼具有α-酮庚二酸轉胺酶活性之核酸序列特別包含根據序列ID 2、序列ID 8、序列ID 12、序列ID 15、序列ID 17、序列ID 19、序列ID 21、序列ID 23、序列ID 25、序列ID 27、序列ID 29或其同系物之胺基酸序列。其中一種或多種核酸序列可形成一種或多種重組載體之一部分。

[0131]於一特佳實施例中，根據本發明之宿主細胞包含編碼具有α-胺庚二酸2-去氫酶活性之胺基酸序列及編碼具有α-胺庚二酸去羧酶活性之胺基酸序列。

[0132]於特佳實施例中，根據本發明之宿主細胞包含編碼具有6-胺己二酸6-去氫酶活性之酶之核酸序列及編碼具有α-酮庚二酸去羧酶活性之酶之核酸序列。

[0133]根據本發明之宿主細胞或載體之一種或多種適當基因特別係選自於前文說明之酶之編碼基因。

[0134]於特定實施例中，宿主細胞爲包含選自於由下列所組成之組群中之至少一個核酸序列之重組細胞：以序列ID 1、序列ID 3、序列ID 4、序列ID 6、序列ID 7、序列ID 11、序列ID 13、序列ID 14、序列ID 16、序列ID 18、序列ID 20、序列ID 22、序列ID 24、序列ID 26、序列ID 28、序列ID 30、序列ID 32、序列ID 33、序列ID 35、序列ID 36、序列ID 38、序列ID 39、序列ID 41、序列ID 42、序列ID 44、序列ID 45、序列ID 47、序列ID 64、序列ID 66、序列ID 68及其功能類似物中之任一者識別之序列。

[0135]編碼具有5-FVA轉胺酶活性之酶之核酸序列特別爲選自於由序列ID 1、3、4、6、7、64、66、68及任何此等序列之功能類似物中之任一者所表示之序列所組成之組群之一序列。

[0136]如此處使用，「功能類似物」一詞至少包括編碼具有相同胺基酸序列之酶之其它序列及編碼此等酶之同系物之其它序列。

[0137]編碼具有AKP去羧酶活性之酶之核酸序列特別爲選自於由序列ID 30、32、33、35、36、38、39、41、42、44、45、47及任何此等序列之功能類似物中之任一者所表示之序列所組成之組群中之一序列。

[0138]於較佳實施例中，宿主細胞包含編碼可催化AAP轉成AKP之轉化作用之酶之核酸序列，該核酸序列係根據序列ID 1、3、7、11、13、14、16、18、20、22、24、26、28、或其功能類似物且可爲野生型序列或非野生型序列。

[0139]於特定實施例中，宿主細胞包含編碼具有α-胺庚二酸去羧酶活性之生物催化劑之至少一個核酸序列，該序列對該宿主細胞可爲同源或非同源。特別此種生物催化劑可選自於由去羧酶(E.C. 4.1.1)所組成之組群，更特別係選自於由下列所組成之組群：麩胺酸去羧酶(EC 4.1.1.15)、二胺庚二酸去羧酶(EC 4.1.1.20)、天冬酸1-去羧酶(EC 4.1.1.11)、分支鏈α-酮酸去羧酶、α-酮異戊酸去羧酶、α-酮戊二酸去羧酶、丙酮酸去羧酶(EC 4.1.1.1)及草醯乙酸去羧酶(EC 4.1.1.3)。

[0140]於特定實施例中，宿主細胞包含催化由AKG (也參考上文)形成AKP之一種或多種酶。可使用構成離胺酸生物合成之α-胺己二酸路徑之一部分之一種酶系。「酶系」一詞用於此處特別係指單一酶或一組可催化特定轉化反應之酶。此種轉化包含具有已知中間物或未知中間物之一種或多種化學反應，例如AKG轉成AKA或AKA轉成AKP。此種系統可存在於細胞內部或與細胞分離。已知轉胺酶經常有寬廣酶基質範圍。若存在有酶基質時期望降低一種或多種此等酶於宿主細胞內之活性，使得AKA轉成α-胺己二酸(AAA)之轉化活性減低，同時維持其它胺基酸或細胞組分之生物合成之相關催化功能。此外，以不含任何其它酶催化活性將導致AKA轉成非期望副產物之宿主細胞爲佳。

[0141]適合用於利用全細胞生物轉換法製備AAP之較佳宿主細胞中，編碼可催化由α-酮戊二酸製備α-酮庚二酸中之至少一個反應步驟之一種或多種生物催化劑。適當生物催化劑例如爲於討論AKP之製備時所述者。

[0142]宿主細胞可選自細菌、酵母或真菌。特定言之，宿主細胞可選自於選自於由麴菌屬、青黴屬、酵母屬、克魯維酵母屬、畢赤酵母屬、假絲酵母屬、漢遜酵母屬、芽胞桿菌屬、棒桿菌屬、假單胞菌屬、葡萄桿菌屬(Gluconobacter)、甲烷球菌屬、甲烷桿菌屬、甲烷暖球菌屬及甲烷八聯球菌屬及埃希氏菌屬等菌屬所組成之組群。此處通常已經選殖且表現一種或多種如前文說明之編碼核酸序列。

[0143]特定言之，宿主細胞以及如此適合用於6-ACA之生化合成之宿主細胞可選自於由下列宿主細胞所組成之組群：大腸桿菌、枯草桿菌、分解澱粉芽胞桿菌(Bacillus amyloliquefaciens)、麩胺酸棒桿菌(Corynebacterium glutamicum)、黑麴菌(Aspergillus niger)、產黃青黴(Penicillium chrysogenum)、釀酒酵母、多形性漢遜酵母(Hansenula polymorpha)、白色念珠菌(Candida albicans)、乳酸克魯維酵母(Kluyveromyces lactis)、短柄畢赤酵母(Pichia stipitis)、巴氏畢赤酵母(Pichia pastoris)、自動趨熱甲烷芽胞桿菌(Methanobacterium thermoautothrophicum)ΔH、馬氏甲烷球菌(Methanococcus maripaludis)、伏氏甲烷球菌(Methanococcus voltae)、阿氏甲烷球菌(Methanosarcina acetivorans)、巴氏甲烷八聯球菌(Methanosarcina barker)及梅氏甲烷八聯球菌(Methanosarcina mazei)等宿主細胞。於較佳實施例中，宿主細胞可製造離胺酸(呈前驅物)。

[0144]宿主細胞原則上爲天然出現之有機體或可爲經基因改造之有機體。此種有機體可使用技藝界已知之突變篩選策略或代謝基因改造策略進行基因改造。於特定實施例中，宿主細胞天然包含(或可製造)適合用於催化本發明方法中之反應步驟之一種或多種酶，諸如選自於由可催化本發明方法中之反應步驟之去羧酶、轉胺酶及胺基酸去氫酶所組成之組群中之一種或多種活性。舉例言之，大腸桿菌天然可製造可催化本發明方法中之轉胺化反應之酶。也可能提供一種重組宿主細胞其帶有兩種重組基因，一種重組

基因編碼可催化本發明方法之一反應步驟之轉胺酶或胺基酸去氫酶，及一種重組基因編碼可催化本發明方法之反應步驟之去羧酶基因。

[0145]例如，宿主細胞可選自於棒桿菌屬特別爲麩胺酸棒桿菌、腸細菌特別爲大腸桿菌、芽胞桿菌屬特別爲枯草桿菌及甲烷芽胞桿菌(B. methanolicus)、及酵母屬特別爲釀酒酵母。特別適合者爲已經發展用於工業上製造離胺酸之麩胺酸棒桿菌或甲烷芽胞桿菌。

[0146]本發明進一步係關於一種微生物，該微生物可爲由天然環境所分離之野生型微生物或重組微生物，包含如選自於由序列ID No. 3、序列ID No. 6、序列ID No. 13、序列ID No. 32、序列ID No. 35、序列ID No. 41、序列ID No. 44、序列ID No. 47及其功能類似物所組成之組群之任一種序列ID識別的核酸序列之DNA。

[0147]如此處所述核苷酸序列之功能類似物特別爲編碼與該核苷酸序列編碼相同胺基酸序列或編碼該核苷酸序列之同系物之該等核苷酸序列。特定言之，較佳功能類似物爲具有與被稱作爲其功能類似物之核苷酸序列於感興趣的宿主細胞內有類似的、相同的或更佳的表現程度之核苷酸序列。

[0148]本發明進一步係關於一種多核苷酸或載體包含以選自於由序列ID No. 3、序列ID No. 6、序列ID No. 13、序列ID No. 32、序列ID No. 35、序列ID No. 41、序列ID No. 44、序列ID No. 47及其非野生型功能類似物所組成之組群

中之任一個序列ID識別之核酸序列。此種多核苷酸或載體比較相對應之野生型基因可更優異地提供宿主細胞，特別爲大腸桿菌宿主細胞，或其它可以高產率催化AKP轉成6-ACA之轉化過程中之至少一個轉化步驟之其它宿主細胞。

[0149]任選地，多核苷酸或載體包含一個或多個核酸序列其編碼適合用於催化根據本發明方法中之反應步驟之一種或多種其它生物催化劑，特別爲前文說明之此等催化劑中之一者或多者。

[0150]本發明進一步係關於一種用於製備α-胺庚二酸(AAP)之方法，包含將AKP轉成AAP，該轉化係藉生物催化劑催化。

[0151]用於此等方法，特別可使用如前文說明具有轉胺酶活性或還原胺化活性之生物催化劑。

[0152]如前文指示，隨後AAP可用於6-ACA之製備。另外，AAP可就此使用，例如用作爲生物化學研究之化學品或用作爲pH緩衝化合物，例如用於製備性分離技術或分析性分離技術諸如液相層析術或毛細電泳。

[0153]又復，於本發明方法所製備之AAP進一步可用於其它化合物之製備，例如AAP可轉成己內醯胺。如前文說明，基於如下實例中舉例說明，AAP可以化學方式例如暴露於高溫而轉成己內醯胺。不欲受理論所限，預期於本反應中，6-ACA可能形成爲短命中間物。

[0154]其次將藉下列實例舉例說明本發明。

實例

概略方法

分子及遺傳技術

[0155]標準遺傳及分子生物學技術爲技藝界概略已知且如前文說明(Maniatis等人，1982年，「分子選殖：實驗室手冊」，冷泉港實驗室，冷泉港，紐約；Miller 1972年「分子遺傳學實驗」，冷泉港實驗室，冷泉港；Sambrook及Russell 2001「分子選殖：實驗室手冊」(第3版)，冷泉港實驗室，冷泉港實驗室出版社；F. Ausubel等人編輯，「分子生物學之流行方案」，格林出版社及威利科技公司，紐約1987)。

質體及種系

[0156]pBAD/Myc-His C係得自茵維基因公司(Invitrogen) (美國加州卡斯貝)。如WO2005/068643所述組成之質體pBAD/Myc-His-DEST用於蛋白質表現。大腸桿菌TOP10 (茵維基因公司，美國加州卡斯貝)用於全部選殖程序及用於標靶基因的表現。

培養基

[0157]LB培養基(10克/升胰蛋白腖，5克/升酵母萃取物，5克/升NaCl)用於大腸桿菌之生長。補充抗生素(50微克/毫升卡卞西林(carbenicillin))來維持質體。爲了於pBAD/Myc-His-DEST所衍生的質體中於P_{BAD}啓動基因之控制之下誘導基因表現，添加L-阿拉伯糖至0.2% (w/v)終濃度。

質體之識別

[0158]攜帶不同基因之質體係藉技藝界一般已知之遺傳學、生物化學、及/或表現型手段加以識別，諸如轉形株對抗生素之抗性、轉形株之PCR診斷分析或質體DNA之純化、已純化的質體DNA之限剪分析或DNA序列分析。

用於測定5-FVA之HPLC-MS分析方法

[0159]經由選擇性反應監視(SRM)-MS，測量變遷129→83而檢測5-FVA。經由測量約6分鐘洗提出之5-FVA峰之峰面積，計算5-FVA濃度。使用外部標準程序進行校準。全部LC-MS實驗皆係於艾吉蘭(Agilent) 1200 LC系統進行，該系統包含第四級幫浦、自動取樣器及柱狀烤爐耦合艾吉蘭6410 QQQ三重四元MS。

LC條件：

[0160]管柱：50 x 4.6毫米紐奎席爾(Nucleosil) C18，5微米(麥肯利及那吉公司(Machery&Nagel))前置管柱耦合至250x4.6毫米內徑普維爾(Prevail) C18，5微米(艾爾科技公司(Alltech))。

管柱溫度： 室溫

洗提劑： A:含0.1%甲酸之水

B:含0.1%甲酸之乙腈

梯度：

時間(分鐘)	%洗提劑B
0	10
6	50
6.1	10
11	10

流速： 1.2毫升/分鐘，於進入MS前流量以1:3分裂。

注入量： 2微升

MS條件：

離子化： 負離子電噴灑

來源條件：離子噴灑電壓：5kV

溫度：350℃

分段器電壓及碰撞能最佳化

掃描模式： 選擇反應模式：變遷m/z 129→83

用於測定AAP之HPLC-MS分析

[0161]藉選定之離子監視(SIM)-MS，測量具有m/z 176之AAP之質子化分子而檢測AAP。經由測量於樣本中於2.7分鐘滯留時間洗提出之AAP峰之尖峰面積而計算AAP濃度。經由使用外部標準程序進行校準。全部LC-MS實驗皆係於由第四級幫浦、除氣器、自動取樣器及柱狀烤爐所組成之艾吉蘭1100 LC系統耦合API 2000三重四元MS (應用生物系統公司(Applied Biosystems))進行。

LC條件如下：

管柱： 50*4紐奎席爾C18，5微米(麥肯利-那吉公司)+250x4.6普維爾C18，5微米(艾爾科技公司)，皆係於室溫(RT)。

洗提劑：A=0.1% (v/v)甲酸於超純水

B=0.1% (v/v)甲酸於乙腈(pa，默克公司(Merck))

流速： 1.2毫升/分鐘，進入MS之前流量以1:3分裂。

梯度： 梯度係於t=0分鐘以90%(v/v)A開始，6分鐘以內改成50% (v/v)A。於6.1分鐘時梯度改成原先條件。

注入量：2微升

MS條件：正離子電噴灑用於離子化。

檢測：　SIM模式於m/z 176，滯留時間100毫秒。

用於測定6-ACA之HPLC-MS分析

[0162]校準：

藉6-ACA之外部校準線(m/z 132→m/z 114，室溫7.5分鐘)進行校準。全部LC-MS實驗皆係於艾吉蘭1100 進行，該儀器裝配有第四級幫浦、除氣器、自動取樣器、柱狀烤爐、及單一四元MS (艾吉蘭，德國沃波恩)。LC-MS條件爲：

管柱：　50*4紐奎席爾(麥肯利-那吉公司) + 250 x 4.6 普維爾C18 (艾爾科技公司)，皆係於室溫(RT)。

洗提劑：A=0.1%(v/v)甲酸於超純水

B=乙腈(pa，默克公司)

流速：　1.0毫升/分鐘，進入MS前流量以1:3分裂。

梯度：　於t=0分鐘梯度始於100% (v/v) A，維持15分鐘，於15分鐘內改成80%(v/v) B (t=30分鐘)。由30至31分鐘，梯度維持恆定於80% (v/v) B。

注入量：5微升

MS檢測：ESI(+)-MS

電噴灑離子化(ESI)係於正掃描模式以下列條件進行：m/z 50-500、50V分段器、0.1 m/z 階梯大小、350℃乾燥氣體溫度、10升氮氣/分鐘乾燥氣體、50 psig霧化器壓力及2.5毛細

電壓。

標靶基因之選殖

表現組成體之設計

[0163]attB位置添加至核糖體結合位置及起始密碼子上游及起始密碼子下游之全部基因來協助使用嘉衛(Gateway)技術(茵維基因公司，美國加州卡斯貝)選殖。

質體之基因合成與組成

[0164]合成基因係得自經最佳化可用於根據DNA2.0之標準程序而於大腸桿菌表現之DNA2.0及密碼子。分別編碼河流弧菌JS17 ω-轉胺酶之胺基酸序列[SEQ ID No.2]及韋氏芽胞桿菌KBAB4轉胺酶之胺基酸序列(ZP_01186960) [SEQ ID No.5]之得自河流弧菌JS17 [SEQ ID No.1]及韋氏芽胞桿菌KBAB4 [SEQ ID No.4]之轉胺酶基因經過密碼子最佳化，所得序列[SEQ ID No.3]及[SEQ ID No.6]係藉DNA合成獲得。

[0165]分別編碼河流弧菌JS17 ω-轉胺酶之胺基酸序列[SEQ ID No.3]、韋氏芽胞桿菌KBAB4轉胺酶(ZP_01186960) [SEQ ID No.6]、大腸桿菌二胺庚二酸去羧酶LysA [SEQ ID No.31]、釀酒酵母丙酮酸去羧酶Pdc [SEQ ID No.34]、活動發酵單胞菌丙酮酸去羧酶Pdc1472A [SEQ ID No.37]、乳酸乳桿菌分支鏈α-酮酸去羧酶KdcA [SEQ ID No.40]及α-酮異戊酸去羧酶KivD [SEQ ID No.43]、及結核分枝桿菌α-酮戊二酸去羧酶Kgd之胺基酸序列[SEQ ID No.46]之得自大腸桿菌[SEQ ID No.30]、釀酒酵母[SEQ ID No.33]、活動發酵

單胞菌[SEQ ID No.36]、乳酸乳桿菌[SEQ ID No.39]、[SEQ ID No.42]、及結核分枝桿菌[SEQ ID No.45]之去羧酶基因也經密碼子最佳化，所得序列[SEQ ID No.32]、[SEQ ID No.35]、[SEQ ID No.38]、[SEQ ID No.41]、[SEQ ID No.44]、及[SEQ ID No.47]分別係藉DNA合成獲得。

[0166]基因組成體如製造商方案(www.invitrogen.com)所述，透過所導入之attB位置及pDONR201 (茵維基因公司)作爲進入載體，使用嘉衛技術(茵維基因公司)選殖入pBAD/Myc-His-DEST表現載體。藉此分別獲得表現載體pBAD-Vfl_AT及pBAD-Bwe_AT。經由使用個別的pBAD表現載體轉形化學勝任大腸桿菌TOP10 (茵維基因公司)，獲得相對應之表現種系。

藉PCR選殖

[0167]編碼生物催化劑之多種基因根據製造商的規格，使用下表所列舉之引子，使用PCR超混合機高可靠度(PCR Supermix High Fidelity) (茵維基因公司)，藉PCR而由基因體DNA擴增。

表2

基因來源	基因序列 ID	酶序列 ID	*引子序列 ID*
綠膿桿菌	7	8	9&10
綠膿桿菌	26	27	60&61
綠膿桿菌	66	67	72&73
綠膿桿菌	68	69	74&75
枯草桿菌	14	15	48&49
枯草桿菌	16	17	50&51
枯草桿菌	64	65	70&71

球狀紅細菌	18	19	52&53
嗜肺性退伍軍人症桿菌(Legionella pneumophilia)	20	21	54&55
歐洲亞硝酸菌(Nitrosomas europaea)	22	23	56&57
淋病奈瑟氏菌(Neisseria gonorrhoeae)	24	25	58&59
沼澤紅假單胞菌(Rhodopseudomonas palustris)	28	29	62&63

[0168]PCR反應係藉瓊脂糖凝膠電泳分析，具有正確尺寸之PCR產物係使用奎克(QIAquick) PCR純化套件組(奎金公司(Qiagen)，德國希爾登)而由凝膠洗提出。如製造商方案所述，透過所導入之attB位置及pDONR-zeo (茵維基因公司)作爲進入載體，使用嘉衛技術(茵維基因公司)將已純化之PCR產物選殖入pBAD/Myc-His-DEST表現載體。藉PCR選殖之基因序列係藉DNA定序加以證實。藉此方式獲得表現載體pBAD-Pae_gi9946143_AT、pBAD-Bsu_gi16078032_AT、pBAD-Bsu_gi16080075_AT、pBAD-Bsu_gi16077991_AT、pBAD-Rsp_AT、pBAD-Lpn_AT、pBAD-Neu_AT、pBAD-Ngo_AT、pBAD-Pae_gi9951299_AT、pBAD-Pae_gi9951072_AT、pBAD-Pae_gi9951630_AT及pBAD-Rpa_AT。相對應之表現種系係藉以pBAD組成體轉形化學上勝任的大腸桿菌TOP10(茵維基因公司)獲得。

用於蛋白質表現之大腸桿菌之生長

[0169]小規模生長係於96深孔孔板使用含0.02% (w/v) L-阿拉伯糖之940微升培養基進行。藉將得自冷凍備用培養之細胞使用96孔衝壓機(庫那公司(Kühner)，瑞士博費登)轉

移細胞進行接種。孔板於軌道振搖機(300 rpm，5厘米振幅)於25℃培養48小時。典型達到OD_{620nm}爲2-4。

細胞溶解物之製備

溶解緩衝液之製備

[0170]溶解緩衝液含有下列成分：

表3

1M MOPS pH 7.5	*5毫升*
DNAse I等級II (羅氏公司(Roche))	*10毫克*
溶菌酶	*200毫克*
$MgSO_4 \cdot 7H_2O$	*123.2毫克*
二硫代蘇糖醇(DTT)	*154.2毫克*
H_2O(米立可公司(MilliQ))	*平衡至100毫升*

溶液係恰於使用前製備。

藉溶解製備不含細胞之萃取物

[0171]藉離心收穫得自小規模生長(參考前段)之細胞，拋棄上清液。離心期間所形成之細胞丸粒於-20℃至少冷凍16小時然後於冰上解凍。500微升剛製妥的溶解緩衝液添加至各孔，藉激烈渦旋孔板2-5分鐘而讓細胞再懸浮。爲了達成細胞溶解，孔板於室溫培養30分鐘。爲了去除細胞殘骸，孔板於4℃及6000g離心20分鐘。上清液移至新的孔板且維持於冰上直到進一步供使用。

藉音振處理製備不含細胞之萃取物

[0172]得自培養基規模生長之細胞(參考前段)藉離心收穫及拋棄上清液。1毫升磷酸鉀緩衝液pH 7添加至0.5克濕細胞丸粒，藉激烈渦旋再度懸浮細胞。爲了達成細胞溶解，細胞經音振處理20分鐘。爲了去除細胞殘骸，溶解產物於4

℃及6000g離心20分鐘。上清液移至新試管內且於-20℃冷凍至進一步供使用。

藉5-甲醯戊酸甲酯之化學水解製備5-甲醯戊酸

[0173]經由5-甲醯戊酸甲酯之化學水解製備轉胺酶反應用之酶基質亦即5-甲醯戊酸如下：10% (w/v) 5-甲醯戊酸甲酯於水溶液使用氫氧化鈉固定於pH 14.1。於20℃培養24小時後，使用鹽酸將pH固定於7.1。

5-甲醯戊酸轉成6-ACA之酶催化反應

[0174]除非另行規定，製備反應混合物包含10 mM 5-甲醯戊酸，20 mM外消旋α-甲基苄基胺，及200 μM吡哆醛5'-磷酸於50 mM磷酸鉀緩衝液，pH 7.0。100微升反應混合物配送至孔板之各孔。爲了引發反應，20微升不含細胞之萃取物添加至各孔。反應混合物於37℃振搖器上培養24小時。此外，化學空白組混合物(不含無細胞之萃取物)及生物空白組(含pBAD/Myc-His C之大腸桿菌TOP10)於相同條件下培養。藉HPLC-MS分析樣本。結果摘述於下表。

表4：於轉胺酶存在下由5-FVA形成6-ACA

生物催化劑	*6-ACA濃度[毫克/千克]*
大腸桿菌TOP10/pBAD-Vfl_AT	*43**
大腸桿菌TOP10/pBAD-Pae_AT	*930*
大腸桿菌TOP10/pBAD-Pae_AT	*25**
大腸桿菌TOP10/pBAD-Bwe_AT	*24**
大腸桿菌TOP10/ pBAD-Bsu_gi16077991_AT	*288*
大腸桿菌TOP10/pBAD-Pae_gi9951072_AT	*1087*
大腸桿菌TOP10/pBAD-Pae_gi9951630_AT	*92*
含pBAD/Myc-His C之大腸桿菌TOP10 (生物空白組)	*0.6*
無(化學空白組)	*n.d.*

n.d.：無法檢測

*該方法之差異在於使用10微升無細胞萃取物替代20微升，吡哆醛5'-磷酸濃度爲50μM而非200μM及孔內反應混合物體積爲190微升而非100微升。

[0175]顯示於轉胺酶存在下由5-FVA形成6-ACA。

AKP轉成5-甲醯戊酸之酶催化反應

[0176]製備反應混合物包含50 mM AKP、5 mM氯化鎂、100 μM吡哆醛5'-磷酸(用於LysA)及1 mM噻胺二磷酸(用於全部其它酶)於100 mM磷酸鉀緩衝液、pH 6.5。4毫升反應混合物配送入反應容器。爲了引發反應，添加1毫升藉音振處理所得之無細胞萃取物至各孔。於商用草醯乙酸去羧酶(西革瑪-亞利敘公司(Sigma-Aldrich)產品號碼04878)之情況下使用50單位。反應混合物使用磁力攪拌器於37℃培養48小時。此外，化學空白組混合物(不含無細胞萃取物)及生物空白組(含pBAD/Myc-His C之大腸桿菌TOP10)於相同條件下培養。反應期間得自不同時間點之樣本藉HPLC-MS分析。結果摘述於下表。

表5：於去羧酶存在下由AKP形成5-FVA

生物催化劑	*5-FVA濃度[毫克/千克]*		
	3小時	18小時	*48小時*
大腸桿菌TOP10/pBAD-LysA	150	590	*720*
大腸桿菌TOP10/pBAD-Pdc	1600	1700	*1300*
大腸桿菌TOP10/pBAD-Pdc1472A	2000	2000	*1600*
大腸桿菌TOP10/pBAD-KdcA	3300	2300	*2200*
大腸桿菌TOP10/pBAD-KivD	820	1400	*1500*
草醯乙酸去羧酶	n.d.	6	*10*
含pBAD/Myc-His C之大腸桿菌TOP10(生物空白組)	n.d.	n.d.	*n.d.*

無(化學空白組)	*n.d.*	*n.d.*	*n.d.*

n.d.：無法檢測

[0177]顯示於去羧酶存在下由AKP形成5-FVA。

於重組去羧酶存在下將AKP轉成6-ACA之酶催化反應

[0178]製備反應混合物包含50 mM AKP、5 mM氯化鎂、100 μM吡哆醛5'-磷酸(用於LysA)及1 mM噻胺二磷酸(用於全部其它測試的生物催化劑)於100 mM磷酸鉀緩衝液、pH 6.5。4毫升反應混合物配送入反應容器。爲了引發反應，添加1毫升藉音振處理所得之無細胞萃取物至各孔。反應混合物使用磁力攪拌器於37℃培養48小時。此外，化學空白組混合物(不含無細胞萃取物)及生物空白組(含pBAD/Myc-His C之大腸桿菌TOP10)於相同條件下培養。反應期間得自不同時間點之樣本藉HPLC-MS分析。結果摘述於下表。

表6：於去羧酶存在下由AKP形成6-ACA

生物催化劑	*6-ACA濃度[毫克/千克]*		
	3小時	18小時	*48小時*
大腸桿菌TOP10/pBAD-LysA	n.a.	0.01	*0*
大腸桿菌TOP10/pBAD-Pdc	0.1	0.3	*n.a.*
大腸桿菌TOP10/pBAD-Pdc1472A	0.03	0.1	*0.2*
大腸桿菌TOP10/pBAD-KdcA	0.04	0.1	*0.3*
大腸桿菌TOP10/pBAD-KivD	n.a.	0.3	*0.6*
含pBAD/Myc-His C之大腸桿菌TOP10(生物空白組)	n.d.	n.d.	*n.d.*
無(化學空白組)	*n.d.*	*n.d.*	*n.d.*

n.a.=未分析

n.d.=無法檢測

[0179]顯示於去羧酶存在下由AKP形成6-ACA。預期大腸桿菌含有天然5-FVA轉胺酶活性。

於重組去羧酶及重組轉胺酶存在下AKP轉成6-ACA之酶催化反應

[0180]製備反應混合物包含50 mM AKP、5 mM氯化鎂、100μM吡哆醛5'-磷酸、1 mM噻胺二磷酸及50 mM外消旋α-甲基苄基胺於100 mM磷酸鉀緩衝液、pH 6.5。1.6毫升反應混合物配送入反應容器。爲了引發反應，添加0.2毫升含無細胞萃取物之去羧酶及0.2毫升含無細胞萃取物之轉胺酶至各個反應容器。反應混合物以磁力攪拌器於37℃培養48小時。此外，化學空白組混合物(不含無細胞萃取物)及生物空白組(含pBAD/Myc-His C之大腸桿菌TOP10)於相同條件下培養。反應期間得自不同時間點之樣本藉HPLC-MS分析。結果摘述於下表。

表7：於重組去羧酶及重組轉胺酶存在下由AKP形成6-ACA

	48小時後之6-ACA濃度[毫克/千克]		
AT / DC	大腸桿菌 TOP10/ pBAD-Vfl_AT	大腸桿菌 TOP10/ pBAD-Bwe_AT	*大腸桿菌 TOP10/ pBAD-PAE_gi9946143_AT*
大腸桿菌 TOP10/pBAD-Pdc	183.4	248.9	*117.9*
大腸桿菌 TOP10/pBAD-Pdc1472A	458.5	471.6	*170.3*
大腸桿菌 TOP10/pBAD-KdcA	497.8	497.8	*275.1*
大腸桿菌 TOP10/pBAD-KivD	*510.9*	*510.9*	*314.4*

AT=轉胺酶

DC=去羧酶

[0181]於化學空白組及生物空白組未檢測得任何6-ACA。

[0182]此外，結果顯示比較其中宿主細胞只含重組去羧酶(而未含重組轉胺酶)之實例，6-ACA之轉化率改良。

於釀酒酵母組成用於轉胺酶及去羧酶之表現之質體

[0183]根據製造商規格且使用特定引子[SEQ ID No.76及77]使用福遜(Phusion) DNA聚合酶(芬贊公司(Finnzymes))，藉PCR由pBAD-Vfl_AT [SEQ ID No.3]擴增得自河流弧菌JS17 之編碼河流弧菌JS17 ω-轉胺酶之胺基酸序列[SEQ ID No.2]之轉胺酶基因。

[0184]根據製造商規格且使用特定引子[SEQ ID No.78及79]使用福遜DNA聚合酶(芬贊公司)，藉PCR由pBAD-Pae_AT擴增得自綠膿桿菌之編碼綠膿桿菌轉胺酶[SEQ ID No.8]之轉胺酶基因[SEQ ID No.7]。

[0185]所得PCR產物使用SpeI及BamHI限剪酶轉殖入載體pAKP-41，分別獲得pAKP-79及pAKP-80，今日含有於釀酒酵母gal10啓動基因及釀酒酵母adh2終結基因下的轉胺酶基因。

[0186]根據製造商規格且使用特定引子[SEQ ID No.80及81]使用福遜DNA聚合酶(芬贊公司)，藉PCR由pBAD-Pdc擴增得自釀酒酵母之編碼釀酒酵母丙酮酸去羧酶Pdc [SEQ ID No.34]之去羧酶基因[SEQ ID No.33]。

[0187]根據製造商規格且使用特定引子[SEQ ID No.82

及83]使用福遜DNA聚合酶(芬贊公司)，藉PCR由pBAD-KdcA擴增得自乳酸乳桿菌之編碼乳酸乳桿菌分支鏈α-酮酸去羧酶KdcA [SEQ ID No.40]之去羧酶基因[SEQ ID No.39]。

[0188]所得PCR產物使用AscI及BamHI限剪酶轉殖入載體pAKP-44，分別獲得pAKP-81及pAKP-82，今日含有於釀酒酵母gal2啓動基因及釀酒酵母pma1終結基因下的轉胺酶基因。

[0189]質體pAKP-79及pAKP-80係以SacI及XbaI經限剪酶消化；質體pAKP-81及pAKP-82係以SalI及XbaI經限剪酶消化。SacI/XbaI轉胺酶片段與片段組合SalI/XbaI去羧酶片段成爲釀酒酵母低複本游離基因載體pRS414，以SalI及SacI接受限剪酶消化。

[0190]所得質體爲：

pAKP-85: Pgal10-Pae_AT-Tadh2 Pgal2-Pdc_DC-Tpma1
pAKP-86: Pgal10-Pae_AT-Tadh2 Pgal2-KdcA_DC-Tpma1
pAKP-87: Pgal10-Vfl_AT-Tadh2 Pgal2-Pdc_DC-Tpma1
pAKP-88: Pgal10-Vfl_AT-Tadh2 Pgal2-KdcA_DC-Tpma1

釀酒酵母之轉形及生長

[0191]釀酒酵母種系CEN.PK113-3C根據Gietz及Woods所述方法(Gietz, R.D.及Woods, R.A. (2002))。酵母之轉形係藉Liac/SS載體DNA/PEG方法。酶學方法350:87-96)，以1微克質體DNA轉形。細胞接種於含1x酵母氮鹼基不含胺基酸及2%葡萄糖之瓊脂孔板。

[0192]所得種系於含0.05%葡萄糖及4%半乳糖之凡杜

恩(Verduyn)最低培養基於30℃需氧生長48小時。

無細胞萃取物之製備

[0193]1毫升磷酸鉀緩衝液(pH 7)添加至0.5克細胞丸粒。此混合物添加至直徑0.4-0.5毫米含0.5克玻璃珠之2毫升衣本朵夫(eppendorf)試管內。樣本以衣本朵夫振搖器(IKA VIBRAX-VXR)激烈振搖20秒。所得無細胞萃取物於14000 rpm及4℃離心5分鐘。上清液用於酶活性檢定分析。

於釀酒酵母於共同表現的去羧酶及轉胺酶存在下將AKP轉成6-ACA之酶催化反應

[0194]製備反應混合物，包含50 mM AKP、5 mM氯化鎂、100 μM吡哆醛5'-磷酸、1 mM噻胺二磷酸及50 mM外消旋α-甲基苄基胺於100 mM磷酸鉀緩衝液、pH 6.5。1.6毫升反應混合物配送入反應容器內。爲了起始反應，加入含去羧酶及轉胺酶之得自釀酒酵母之無細胞萃取物0.4毫升至各個反應容器。反應混合物於37℃藉磁力攪拌器培養。此外，化學空白組混合物(不含無細胞萃取物)及生物空白組(釀酒酵母)於相同條件下培養。19小時培養後所得樣本藉HPLC-MS分析。結果摘述於下表。

表8：使用微生物作爲生物催化劑由AKP形成6-ACA

生物催化劑	*6-ACA濃度[毫克/千克]*
釀酒酵母pAKP-85	*63*
釀酒酵母pAKP-86	*226*
釀酒酵母pAKP-87	*1072*
釀酒酵母pAKP-88	*4783*
釀酒酵母(生物空白組)	*3.9*

無(化學空白組)	*1.3*

α-酮庚二酸轉成α-胺庚二酸之酶催化反應

[0195]製備反應混合物，包含10 mM α-酮庚二酸，20 mM L-丙胺酸，及50 μM吡哆醛5'-磷酸於50 mM磷酸鉀緩衝液，pH 7.0。800微升反應混合物配送入孔板之各孔。爲了起始反應，添加200微升細胞溶解物至各孔。反應混合物於37℃於振搖器上培養24小時。此外，化學空白組混合物(不含無細胞之萃取物)及生物空白組(含pBAD/Myc-His C之大腸桿菌TOP10)於相同條件下培養。樣本藉HPLC-MS分析。結果摘述於下表。

表9：於轉胺酶存在下由AKP形成AAP

生物催化劑	*AAP濃度 [毫克/千克] (24小時後)*
大腸桿菌TOP10/pBAD-Vfl_AT	*3.7*
大腸桿菌TOP10/pBAD-Psy_AT	*15.8*
大腸桿菌TOP10/pBAD-Bsu_gi16078032_AT	*11.2*
大腸桿菌TOP10/pBAD-Rsp_AT	*9.8*
大腸桿菌TOP10/pBAD-Bsu_gi16080075_AT	*4.6*
大腸桿菌TOP10/pBAD-Lpn_AT	*5.4*
大腸桿菌TOP10/pBAD-Neu_AT	*7.7*
大腸桿菌TOP10/pBAD-Ngo_AT	*5.1*
大腸桿菌TOP10/pBAD-Pae_gi9951299_AT	*5.6*
大腸桿菌TOP10/pBAD-Rpa_AT	*5.4*
含pBAD/Myc-His C之大腸桿菌TOP10 (生物空白組)	*1.4*
無(化學空白組)	*0*

[0196]顯示由AKP形成AAP係藉生物催化劑催化。

AAP之化學轉化成己內醯胺

[0197]於1.5克D,L-2-胺庚二酸於21毫升環己酮之懸浮液內加入0.5毫升環己烯酮。混合物於由約回流加熱20小時(約160℃)。冷卻至室溫後，反應混合物經傾析，於減壓下蒸發去除澄清溶液。剩餘2克褐色油藉^{1}H-NMR及HPLC分析，含有0.8 wt%己內醯胺及6 wt%己內醯胺之環狀寡聚物。

【符號說明】

(無)

【生物材料寄存】

(無)

【序列表】

<110> DSM 智慧財產有限公司

<120> 由α-酮庚二酸製備 6-胺己酸之技術

<130> P84198PC00

<150> EP 08152584.2
<151> 2008-03-11

<160> 83

<170> PatentIn version 3.3

<210> 1
<211> 1362
<212> DNA
<213> 河流弧菌

<220>
<221> CDS
<222> (1)..(1362)

<400> 1

```
atg aac aaa ccg caa agc tgg gaa gcc cgg gcc gag acc tat tcg ctc      48
Met Asn Lys Pro Gln Ser Trp Glu Ala Arg Ala Glu Thr Tyr Ser Leu
1               5                  10                  15

tat ggt ttc acc gac atg cct tcg ctg cat cag cgc ggc acg gtc gtc      96
Tyr Gly Phe Thr Asp Met Pro Ser Leu His Gln Arg Gly Thr Val Val
            20                  25                  30

gtg acc cat ggc gag gga ccc tat atc gtc gat gtg aat ggc cgg cgt     144
Val Thr His Gly Glu Gly Pro Tyr Ile Val Asp Val Asn Gly Arg Arg
        35                  40                  45

tat ctg gac gcc aac tcg ggc ctg tgg aac atg gtc gcg ggc ttt gac     192
Tyr Leu Asp Ala Asn Ser Gly Leu Trp Asn Met Val Ala Gly Phe Asp
    50                  55                  60

cac aag ggg ctg atc gac gcc gcc aag gcc caa tac gag cgt ttt ccc     240
His Lys Gly Leu Ile Asp Ala Ala Lys Ala Gln Tyr Glu Arg Phe Pro
65                  70                  75                  80

ggt tat cac gcc ttt ttc ggc cgc atg tcc gat cag acg gta atg ctg     288
Gly Tyr His Ala Phe Phe Gly Arg Met Ser Asp Gln Thr Val Met Leu
                85                  90                  95

tcg gaa aag ctg gtc gag gtg tcg ccc ttt gat tcg ggc cgg gtg ttc     336
Ser Glu Lys Leu Val Glu Val Ser Pro Phe Asp Ser Gly Arg Val Phe
            100                 105                 110

tat aca aac tcg ggg tcc gag gcg aat gac acc atg gtc aag atg cta     384
Tyr Thr Asn Ser Gly Ser Glu Ala Asn Asp Thr Met Val Lys Met Leu
        115                 120                 125
```

```
tgg ttc ctg cat gca gcc gag ggc aaa ccg caa aag cgc aag atc ctg      432
Trp Phe Leu His Ala Ala Glu Gly Lys Pro Gln Lys Arg Lys Ile Leu
    130                 135                 140

acc cgc tgg aac gcc tat cac ggc gtg acc gcc gtt tcg gcc agc atg      480
Thr Arg Trp Asn Ala Tyr His Gly Val Thr Ala Val Ser Ala Ser Met
145                 150                 155                 160

acc ggc aag ccc tat aat tcg gtc ttt ggc ctg ccg ctg ccg ggc ttt      528
Thr Gly Lys Pro Tyr Asn Ser Val Phe Gly Leu Pro Leu Pro Gly Phe
                165                 170                 175

gtg cat ctg acc tgc ccg cat tac tgg cgc tat ggc gaa gag ggc gaa      576
Val His Leu Thr Cys Pro His Tyr Trp Arg Tyr Gly Glu Glu Gly Glu
            180                 185                 190

acc gaa gag cag ttc gtc gcc cgc ctc gcc cgc gag ctg gag gaa acg      624
Thr Glu Glu Gln Phe Val Ala Arg Leu Ala Arg Glu Leu Glu Glu Thr
        195                 200                 205

atc cag cgc gag ggc gcc gac acc atc gcc ggt ttc ttt gcc gaa ccg      672
Ile Gln Arg Glu Gly Ala Asp Thr Ile Ala Gly Phe Phe Ala Glu Pro
    210                 215                 220

gtg atg ggc gcg ggc ggc gtg att ccc ccg gcc aag ggc tat ttc cag      720
Val Met Gly Ala Gly Gly Val Ile Pro Pro Ala Lys Gly Tyr Phe Gln
225                 230                 235                 240

gcg atc ctg cca atc ctg cgc aaa tat gac atc ccg gtc atc tcg gac      768
Ala Ile Leu Pro Ile Leu Arg Lys Tyr Asp Ile Pro Val Ile Ser Asp
                245                 250                 255

gag gtg atc tgc ggt ttc gga cgc acc ggt aac acc tgg ggc tgc gtg      816
Glu Val Ile Cys Gly Phe Gly Arg Thr Gly Asn Thr Trp Gly Cys Val
            260                 265                 270

acc tat gac ttt aca ccc gat gca atc atc tcg tcc aag aat ctt aca      864
Thr Tyr Asp Phe Thr Pro Asp Ala Ile Ile Ser Ser Lys Asn Leu Thr
        275                 280                 285

gcg ggc ttt ttc ccc atg ggg gcg gtg atc ctt ggc ccg gaa ctt tcc      912
Ala Gly Phe Phe Pro Met Gly Ala Val Ile Leu Gly Pro Glu Leu Ser
    290                 295                 300

aaa cgg ctg gaa acc gca atc gag gcg atc gag gaa ttc ccc cat ggc      960
Lys Arg Leu Glu Thr Ala Ile Glu Ala Ile Glu Glu Phe Pro His Gly
305                 310                 315                 320

ttt acc gcc tcg ggc cat ccg gtc ggc tgt gct att gcg ctg aaa gca     1008
Phe Thr Ala Ser Gly His Pro Val Gly Cys Ala Ile Ala Leu Lys Ala
                325                 330                 335

atc gac gtg gtg atg aat gaa ggg ctg gct gag aac gtc cgc cgc ctt     1056
Ile Asp Val Val Met Asn Glu Gly Leu Ala Glu Asn Val Arg Arg Leu
            340                 345                 350

gcc ccc cgt ttc gag gaa agg ctg aaa cat atc gcc gag cgc ccg aac     1104
Ala Pro Arg Phe Glu Glu Arg Leu Lys His Ile Ala Glu Arg Pro Asn
```

```
          355                 360                 365

atc ggt gaa tat cgc ggc atc ggc ttc atg tgg gcg ctg gag gct gtc      1152
Ile Gly Glu Tyr Arg Gly Ile Gly Phe Met Trp Ala Leu Glu Ala Val
    370                 375                 380

aag gac aag gca agc aag acg ccg ttc gac ggc aac ctg tcg gtc agc      1200
Lys Asp Lys Ala Ser Lys Thr Pro Phe Asp Gly Asn Leu Ser Val Ser
385                 390                 395                 400

gag cgt atc gcc aat acc tgc acc gat ctg ggg ctg att tgc cgg ccg      1248
Glu Arg Ile Ala Asn Thr Cys Thr Asp Leu Gly Leu Ile Cys Arg Pro
                405                 410                 415

ctt ggt cag tcc gtc gtc ctt tgt ccg ccc ttt atc ctg acc gag gcg      1296
Leu Gly Gln Ser Val Val Leu Cys Pro Pro Phe Ile Leu Thr Glu Ala
            420                 425                 430

cag atg gat gag atg ttc gat aaa ctc gaa aaa gcc ctt gat aag gtc      1344
Gln Met Asp Glu Met Phe Asp Lys Leu Glu Lys Ala Leu Asp Lys Val
        435                 440                 445

ttt gcc gag gtt gcc tga                                             1362
Phe Ala Glu Val Ala
    450
```

<210> 2
<211> 453
<212> PRT
<213> 河流弧菌

<400> 2

```
Met Asn Lys Pro Gln Ser Trp Glu Ala Arg Ala Glu Thr Tyr Ser Leu
1               5                   10                  15

Tyr Gly Phe Thr Asp Met Pro Ser Leu His Gln Arg Gly Thr Val Val
            20                  25                  30

Val Thr His Gly Glu Gly Pro Tyr Ile Val Asp Val Asn Gly Arg Arg
        35                  40                  45

Tyr Leu Asp Ala Asn Ser Gly Leu Trp Asn Met Val Ala Gly Phe Asp
    50                  55                  60

His Lys Gly Leu Ile Asp Ala Ala Lys Ala Gln Tyr Glu Arg Phe Pro
65                  70                  75                  80

Gly Tyr His Ala Phe Phe Gly Arg Met Ser Asp Gln Thr Val Met Leu
                85                  90                  95

Ser Glu Lys Leu Val Glu Val Ser Pro Phe Asp Ser Gly Arg Val Phe
```

100 105 110

Tyr Thr Asn Ser Gly Ser Glu Ala Asn Asp Thr Met Val Lys Met Leu
115 120 125

Trp Phe Leu His Ala Ala Glu Gly Lys Pro Gln Lys Arg Lys Ile Leu
130 135 140

Thr Arg Trp Asn Ala Tyr His Gly Val Thr Ala Val Ser Ala Ser Met
145 150 155 160

Thr Gly Lys Pro Tyr Asn Ser Val Phe Gly Leu Pro Leu Pro Gly Phe
165 170 175

Val His Leu Thr Cys Pro His Tyr Trp Arg Tyr Gly Glu Glu Gly Glu
180 185 190

Thr Glu Glu Gln Phe Val Ala Arg Leu Ala Arg Glu Leu Glu Glu Thr
195 200 205

Ile Gln Arg Glu Gly Ala Asp Thr Ile Ala Gly Phe Phe Ala Glu Pro
210 215 220

Val Met Gly Ala Gly Gly Val Ile Pro Pro Ala Lys Gly Tyr Phe Gln
225 230 235 240

Ala Ile Leu Pro Ile Leu Arg Lys Tyr Asp Ile Pro Val Ile Ser Asp
245 250 255

Glu Val Ile Cys Gly Phe Gly Arg Thr Gly Asn Thr Trp Gly Cys Val
260 265 270

Thr Tyr Asp Phe Thr Pro Asp Ala Ile Ile Ser Ser Lys Asn Leu Thr
275 280 285

Ala Gly Phe Phe Pro Met Gly Ala Val Ile Leu Gly Pro Glu Leu Ser
290 295 300

Lys Arg Leu Glu Thr Ala Ile Glu Ala Ile Glu Glu Phe Pro His Gly
305 310 315 320

Phe Thr Ala Ser Gly His Pro Val Gly Cys Ala Ile Ala Leu Lys Ala
325 330 335

Ile Asp Val Val Met Asn Glu Gly Leu Ala Glu Asn Val Arg Arg Leu
340 345 350

Ala Pro Arg Phe Glu Glu Arg Leu Lys His Ile Ala Glu Arg Pro Asn
355 360 365

Ile Gly Glu Tyr Arg Gly Ile Gly Phe Met Trp Ala Leu Glu Ala Val
370 375 380

Lys Asp Lys Ala Ser Lys Thr Pro Phe Asp Gly Asn Leu Ser Val Ser
385 390 395 400

Glu Arg Ile Ala Asn Thr Cys Thr Asp Leu Gly Leu Ile Cys Arg Pro
405 410 415

Leu Gly Gln Ser Val Val Leu Cys Pro Pro Phe Ile Leu Thr Glu Ala
420 425 430

Gln Met Asp Glu Met Phe Asp Lys Leu Glu Lys Ala Leu Asp Lys Val
435 440 445

Phe Ala Glu Val Ala
450

<210> 3
<211> 1362
<212> DNA
<213> 人造

<220>
<223> 河流弧菌 JS17 ω-轉胺苷密碼子最佳化基因

<400> 3
atgaataaac cacagtcttg ggaagctcgt gctgaaacct atagcctgta cggctttacc 60

gatatgccgt ctctgcacca gcgtggtact gtagtggtaa cgcacggtga gggcccgtac 120

atcgtggacg ttaatggccg ccgttacctg gatgcaaaca gcggcctgtg gaacatggtt 180

gcgggcttcg accacaaagg cctgatcgat gccgcaaaag cgcagtacga acgcttcccg 240

ggttatcacg cgttctttgg ccgtatgagc gaccagactg tgatgctgag cgaaaaactg 300

gttgaagtgt ccccgttcga tagcggtcgt gtcttttaca ctaactctgg cagcgaggct 360

aacgatacca tggttaagat gctgtggttc ctgcacgcag cggaaggcaa acctcagaaa 420

cgtaaaattc tgacccgttg gaacgcttat cacggtgtga ctgctgtttc cgcatctatg 480

accggtaaac cgtataacag cgtgttcggt ctgccgctgc ctggcttcgt gcatctgacc 540

tgcccgcact actggcgtta tggtgaggaa ggcgaaactg aggaacagtt cgtggcgcgt 600

ctggctcgtg aactggaaga aaccattcaa cgcgaaggtg cagatactat cgcgggcttc 660

tttgcggagc ctgttatggg tgccggcggt gtgattccgc cggcgaaggg ctatttccag 720

gcaatcctgc cgatcctgcg caagtacgac attccggtta tttctgacga agtgatctgc 780

ggcttcggcc gcaccggtaa cacctggggc tgcgtgacgt atgacttcac tccggacgca 840

atcattagct ctaaaaacct gactgcgggt ttcttcccta tgggcgccgt aatcctgggc 900

ccagaactgt ctaagcgcct ggaaaccgcc atcgaggcaa tcgaagagtt cccgcacggt 960

ttcactgcta gcggccatcc ggtaggctgc gcaatcgcgc tgaaggcgat cgatgttgtc 1020

atgaacgagg gcctggcgga aaacgtgcgc cgcctggcgc cgcgttttga agaacgtctg 1080

aaacacattg ctgagcgccc gaacattggc gaatatcgcg gcatcggttt catgtgggcc 1140

ctggaagcag ttaaagataa agctagcaag accccgttcg acggcaacct gtccgtgagc 1200

gaacgtatcg ctaatacctg tacggacctg ggtctgatct gccgtccgct gggtcagtcc 1260

gtagttctgt gcccaccatt tatcctgacc gaagcgcaga tggatgaaat gttcgataaa 1320

ctggagaaag ctctggataa agtgttcgct gaagtcgcgt aa 1362

<210> 4
<211> 1350
<212> DNA
<213> 韋氏芽胞桿菌

<220>
<221> CDS
<222> (1)..(1350)

<400> 4

```
gtg caa gcg acg gag caa aca caa agt ttg aaa aaa aca gat gaa aag      48
Val Gln Ala Thr Glu Gln Thr Gln Ser Leu Lys Lys Thr Asp Glu Lys
1               5                  10                  15

tac ctt tgg cat gcg atg aga gga gca gcc cct agt cca acg aat tta      96
Tyr Leu Trp His Ala Met Arg Gly Ala Ala Pro Ser Pro Thr Asn Leu
            20                  25                  30

att atc aca aaa gca gaa ggg gca tgg gtg acg gat att gat gga aac     144
Ile Ile Thr Lys Ala Glu Gly Ala Trp Val Thr Asp Ile Asp Gly Asn
        35                  40                  45

cgt tat tta gac ggt atg tcc ggt ctt tgg tgc gtg aat gtt ggg tat     192
Arg Tyr Leu Asp Gly Met Ser Gly Leu Trp Cys Val Asn Val Gly Tyr
    50                  55                  60

ggt cga aaa gaa ctt gca aga gcg gcg ttt gaa cag ctt gaa gaa atg     240
Gly Arg Lys Glu Leu Ala Arg Ala Ala Phe Glu Gln Leu Glu Glu Met
65                  70                  75                  80
```

```
ccg tat ttc cct ctg act caa agt cat gtt cct gct att aaa tta gca      288
Pro Tyr Phe Pro Leu Thr Gln Ser His Val Pro Ala Ile Lys Leu Ala
                85                  90                  95

gaa aaa ttg aat gaa tgg ctt gat gat gaa tac gtc att ttc ttt tct      336
Glu Lys Leu Asn Glu Trp Leu Asp Asp Glu Tyr Val Ile Phe Phe Ser
            100                 105                 110

aac agt gga tcg gaa gcg aat gaa aca gca ttt aaa att gct cgt caa      384
Asn Ser Gly Ser Glu Ala Asn Glu Thr Ala Phe Lys Ile Ala Arg Gln
        115                 120                 125

tat cat caa caa aaa ggt gat cat gga cgc tat aag ttt att tcc cgc      432
Tyr His Gln Gln Lys Gly Asp His Gly Arg Tyr Lys Phe Ile Ser Arg
    130                 135                 140

tac cgc gct tat cac ggt aac tca atg gga gct ctt gca gca aca ggt      480
Tyr Arg Ala Tyr His Gly Asn Ser Met Gly Ala Leu Ala Ala Thr Gly
145                 150                 155                 160

caa gca cag cga aag tat aaa tat gaa cca ctc ggg caa gga ttc ctg      528
Gln Ala Gln Arg Lys Tyr Lys Tyr Glu Pro Leu Gly Gln Gly Phe Leu
                165                 170                 175

cat gta gca ccg cct gat acg tat cga aat cca gag gat gtt cat aca      576
His Val Ala Pro Pro Asp Thr Tyr Arg Asn Pro Glu Asp Val His Thr
            180                 185                 190

ctg gca agt gct gag gaa atc gat cgt gtc atg aca tgg gag tta agc      624
Leu Ala Ser Ala Glu Glu Ile Asp Arg Val Met Thr Trp Glu Leu Ser
        195                 200                 205

caa aca gta gcc ggt gtg att atg gag cca atc att act ggg ggc gga      672
Gln Thr Val Ala Gly Val Ile Met Glu Pro Ile Ile Thr Gly Gly Gly
    210                 215                 220

att tta atg cct cct gat gga tat atg gga aaa gta aaa gaa att tgc      720
Ile Leu Met Pro Pro Asp Gly Tyr Met Gly Lys Val Lys Glu Ile Cys
225                 230                 235                 240

gag aag cac ggt gcg ttg ctc att tgt gat gaa gtt ata tgt gga ttt      768
Glu Lys His Gly Ala Leu Leu Ile Cys Asp Glu Val Ile Cys Gly Phe
                245                 250                 255

ggc cgg aca ggg aag cca ttt gga ttt atg aat tat ggc gtc aaa cca      816
Gly Arg Thr Gly Lys Pro Phe Gly Phe Met Asn Tyr Gly Val Lys Pro
            260                 265                 270

gat atc att aca atg gca aaa ggt att aca agt gcg tat ctt cct ttg      864
Asp Ile Ile Thr Met Ala Lys Gly Ile Thr Ser Ala Tyr Leu Pro Leu
        275                 280                 285

tca gca aca gca gtt aga cga gag gtt tat gag gca ttc gta ggt agt      912
Ser Ala Thr Ala Val Arg Arg Glu Val Tyr Glu Ala Phe Val Gly Ser
    290                 295                 300

gat gat tat gat cgc ttc cgc cat gta aat acg ttc gga ggg aat cct      960
Asp Asp Tyr Asp Arg Phe Arg His Val Asn Thr Phe Gly Gly Asn Pro
```

305 310 315 320

gct gct tgc gct tta gct ttg aag aat tta gaa att atg gag aat gag 1008
Ala Ala Cys Ala Leu Ala Leu Lys Asn Leu Glu Ile Met Glu Asn Glu
325 330 335

aaa ctc att gaa cgt tcc aaa gaa ttg ggt gaa cga ctg tta tat gag 1056
Lys Leu Ile Glu Arg Ser Lys Glu Leu Gly Glu Arg Leu Leu Tyr Glu
340 345 350

cta gag gat gta aaa gag cat cca aac gta ggg gat gtt cgc gga aag 1104
Leu Glu Asp Val Lys Glu His Pro Asn Val Gly Asp Val Arg Gly Lys
355 360 365

ggc ctt ctt tta ggc att gaa cta gtg gaa gat aag caa aca aaa gaa 1152
Gly Leu Leu Leu Gly Ile Glu Leu Val Glu Asp Lys Gln Thr Lys Glu
370 375 380

ccg gct tcc att gaa aag atg aac aaa gtc atc aat gct tgt aaa gaa 1200
Pro Ala Ser Ile Glu Lys Met Asn Lys Val Ile Asn Ala Cys Lys Glu
385 390 395 400

aaa ggt cta att att ggt aaa aat ggt gac act gtc gca ggt tac aat 1248
Lys Gly Leu Ile Ile Gly Lys Asn Gly Asp Thr Val Ala Gly Tyr Asn
405 410 415

aat att ttg cag ctt gca cct cca tta agc atc aca gag gaa gac ttt 1296
Asn Ile Leu Gln Leu Ala Pro Pro Leu Ser Ile Thr Glu Glu Asp Phe
420 425 430

act ttt atc gtt aaa aca atg aaa gaa tgt tta tcc cgc att aac ggg 1344
Thr Phe Ile Val Lys Thr Met Lys Glu Cys Leu Ser Arg Ile Asn Gly
435 440 445

cag taa 1350
Gln

<210> 5
<211> 449
<212> PRT
<213> 韋氏芽胞桿菌

<400> 5

Val Gln Ala Thr Glu Gln Thr Gln Ser Leu Lys Lys Thr Asp Glu Lys
1 5 10 15

Tyr Leu Trp His Ala Met Arg Gly Ala Ala Pro Ser Pro Thr Asn Leu
20 25 30

Ile Ile Thr Lys Ala Glu Gly Ala Trp Val Thr Asp Ile Asp Gly Asn
35 40 45

Arg Tyr Leu Asp Gly Met Ser Gly Leu Trp Cys Val Asn Val Gly Tyr

50 55 60

Gly Arg Lys Glu Leu Ala Arg Ala Ala Phe Glu Gln Leu Glu Glu Met
65 70 75 80

Pro Tyr Phe Pro Leu Thr Gln Ser His Val Pro Ala Ile Lys Leu Ala
85 90 95

Glu Lys Leu Asn Glu Trp Leu Asp Asp Glu Tyr Val Ile Phe Phe Ser
100 105 110

Asn Ser Gly Ser Glu Ala Asn Glu Thr Ala Phe Lys Ile Ala Arg Gln
115 120 125

Tyr His Gln Gln Lys Gly Asp His Gly Arg Tyr Lys Phe Ile Ser Arg
130 135 140

Tyr Arg Ala Tyr His Gly Asn Ser Met Gly Ala Leu Ala Ala Thr Gly
145 150 155 160

Gln Ala Gln Arg Lys Tyr Lys Tyr Glu Pro Leu Gly Gln Gly Phe Leu
165 170 175

His Val Ala Pro Pro Asp Thr Tyr Arg Asn Pro Glu Asp Val His Thr
180 185 190

Leu Ala Ser Ala Glu Glu Ile Asp Arg Val Met Thr Trp Glu Leu Ser
195 200 205

Gln Thr Val Ala Gly Val Ile Met Glu Pro Ile Ile Thr Gly Gly Gly
210 215 220

Ile Leu Met Pro Pro Asp Gly Tyr Met Gly Lys Val Lys Glu Ile Cys
225 230 235 240

Glu Lys His Gly Ala Leu Leu Ile Cys Asp Glu Val Ile Cys Gly Phe
245 250 255

Gly Arg Thr Gly Lys Pro Phe Gly Phe Met Asn Tyr Gly Val Lys Pro
260 265 270

Asp Ile Ile Thr Met Ala Lys Gly Ile Thr Ser Ala Tyr Leu Pro Leu
275 280 285

Ser Ala Thr Ala Val Arg Arg Glu Val Tyr Glu Ala Phe Val Gly Ser
290 295 300

Asp Asp Tyr Asp Arg Phe Arg His Val Asn Thr Phe Gly Gly Asn Pro
305 310 315 320

Ala Ala Cys Ala Leu Ala Leu Lys Asn Leu Glu Ile Met Glu Asn Glu
325 330 335

Lys Leu Ile Glu Arg Ser Lys Glu Leu Gly Glu Arg Leu Leu Tyr Glu
340 345 350

Leu Glu Asp Val Lys Glu His Pro Asn Val Gly Asp Val Arg Gly Lys
355 360 365

Gly Leu Leu Leu Gly Ile Glu Leu Val Glu Asp Lys Gln Thr Lys Glu
370 375 380

Pro Ala Ser Ile Glu Lys Met Asn Lys Val Ile Asn Ala Cys Lys Glu
385 390 395 400

Lys Gly Leu Ile Ile Gly Lys Asn Gly Asp Thr Val Ala Gly Tyr Asn
405 410 415

Asn Ile Leu Gln Leu Ala Pro Pro Leu Ser Ile Thr Glu Glu Asp Phe
420 425 430

Thr Phe Ile Val Lys Thr Met Lys Glu Cys Leu Ser Arg Ile Asn Gly
435 440 445

Gln

<210> 6
<211> 1350
<212> DNA
<213> 人造

<220>
<223> 韋氏芽胞桿菌 KBAB4 轉胺苷密碼子最佳化基因

<400> 6
atgcaggcta ccgaacaaac ccaatctctg aaaaagactg acgaaaaata tctgtggcac 60

gcgatgcgcg gtgcagctcc gtctccgacc aacctgatta ttaccaaagc tgaaggcgcg 120

tgggtgaccg acattgacgg taaccgttat ctggatggca tgagcggcct gtggtgtgtt 180

aatgtcggtt atggccgtaa ggagctggcg cgcgcggcat ttgaacaact ggaagaaatg 240

ccgtacttcc cgctgactca aagccatgtg ccggctatca aactggcgga aaaactgaac 300

gaatggctgg acgacgaata cgtgattttc ttctctaatt ctggctccga agcaaacgaa 360

accgcattca aaatcgcccg tcaatatcac cagcagaaag gtgaccacgg ccgctataaa 420

ttcatcagcc gttatcgtgc ataccatggt aattctatgg gtgcgctggc tgctaccggt 480

caggctcagc gcaaatacaa gtacgaaccg ctgggtcagg gttttctgca cgttgcacca 540

ccggatacct accgtaaccc ggaagacgtc cacaccctgg cttctgccga agaaatcgat 600

cgtgttatga cctgggagct gtcccagact gttgcgggtg ttatcatgga acctattatt 660

accggtggtg gcattctgat gccgccggac ggttatatgg gtaaagtcaa ggaaatctgc 720

gaaaaacacg gcgcgctgct gatctgcgat gaagttatct gtggcttcgg tcgcaccggc 780

aaaccatttg gcttcatgaa ttatggcgta aaacctgaca ttattaccat ggctaaaggc 840

attacttccg cttatctgcc gctgagcgcg accgcagttc gccgcgaagt ttatgaagcg 900

tttgttggtt ctgatgatta cgaccgtttc cgtcatgtaa acacgtttgg cggtaaccca 960

gcggcatgtg cgctggcgct gaaaaacctg gaaatcatgg aaaacgaaaa gctgatcgaa 1020

cgtagcaaag aactgggtga acgtctgctg tacgaactgg aagatgtcaa agaacacccg 1080

aacgtgggcg atgttcgcgg taaaggcctg ctgctgggta ttgaactggt tgaagacaaa 1140

cagaccaagg aaccggcttc cattgaaaag atgaacaaag tgattaacgc gtgcaaagag 1200

aaaggcctga tcattggtaa gaacggtgat accgtggcag gttataacaa cattctgcag 1260

ctggcgccgc ctctgagcat cactgaagaa gatttcacct tcatcgtcaa aactatgaag 1320

gagtgcctga gccgcatcaa tggtcagtaa 1350

<210> 7
<211> 1371
<212> DNA
<213> 綠膿桿菌(gi 9946143)

<220>
<221> CDS
<222> (1)..(1371)

<400> 7

```
atg aac agc caa atc acc aac gcc aag acc cgt gag tgg cag gcg ttg      48
Met Asn Ser Gln Ile Thr Asn Ala Lys Thr Arg Glu Trp Gln Ala Leu
1               5                  10                  15

agc cgc gac cac cat ctg ccg ccg ttc acc gac tac aag cag ttg aac      96
Ser Arg Asp His His Leu Pro Pro Phe Thr Asp Tyr Lys Gln Leu Asn
            20                  25                  30
```

```
gag aag ggc gcg cgg atc atc acc aag gcc gaa ggc gtc tat atc tgg      144
Glu Lys Gly Ala Arg Ile Ile Thr Lys Ala Glu Gly Val Tyr Ile Trp
        35                  40                  45

gac agc gag ggc aac aag atc ctc gat gcg atg gcc ggc ctc tgg tgc      192
Asp Ser Glu Gly Asn Lys Ile Leu Asp Ala Met Ala Gly Leu Trp Cys
    50                  55                  60

gtc aac gtc ggc tac ggc cgc gag gag ctg gtc cag gcc gcc acc cgg      240
Val Asn Val Gly Tyr Gly Arg Glu Glu Leu Val Gln Ala Ala Thr Arg
65                  70                  75                  80

cag atg cgc gag ttg ccg ttc tac aac ctg ttc ttc cag acc gcc cac      288
Gln Met Arg Glu Leu Pro Phe Tyr Asn Leu Phe Phe Gln Thr Ala His
                85                  90                  95

ccg ccg gtg gtc gag ctg gcc aag gcg atc gcc gac gtc gct ccg gaa      336
Pro Pro Val Val Glu Leu Ala Lys Ala Ile Ala Asp Val Ala Pro Glu
            100                 105                 110

ggc atg aac cac gtg ttc ttc acc ggc tcc ggc tcc gag gcc aac gac      384
Gly Met Asn His Val Phe Phe Thr Gly Ser Gly Ser Glu Ala Asn Asp
        115                 120                 125

acc gtg ctg cgt atg gtc cgc cac tat tgg gcg acc aag ggc cag ccg      432
Thr Val Leu Arg Met Val Arg His Tyr Trp Ala Thr Lys Gly Gln Pro
    130                 135                 140

cag aag aaa gtg gtg atc ggc cgc tgg aac ggc tac cac ggc tcc acc      480
Gln Lys Lys Val Val Ile Gly Arg Trp Asn Gly Tyr His Gly Ser Thr
145                 150                 155                 160

gtc gcc ggc gtc agc ctg ggc ggc atg aag gcg ttg cat gag cag ggt      528
Val Ala Gly Val Ser Leu Gly Gly Met Lys Ala Leu His Glu Gln Gly
                165                 170                 175

gat ttc ccc atc ccg ggc atc gtc cac atc gcc cag ccc tac tgg tac      576
Asp Phe Pro Ile Pro Gly Ile Val His Ile Ala Gln Pro Tyr Trp Tyr
            180                 185                 190

ggc gag ggc ggc gac atg tcg ccg gac gag ttc ggc gtc tgg gcc gcc      624
Gly Glu Gly Gly Asp Met Ser Pro Asp Glu Phe Gly Val Trp Ala Ala
        195                 200                 205

gag cag ttg gag aag aag att ctc gaa gtg ggc gag gaa aac gtc gcc      672
Glu Gln Leu Glu Lys Lys Ile Leu Glu Val Gly Glu Glu Asn Val Ala
    210                 215                 220

gcc ttc atc gcc gag ccg atc cag ggc gcc ggc ggc gtg atc gtc ccg      720
Ala Phe Ile Ala Glu Pro Ile Gln Gly Ala Gly Gly Val Ile Val Pro
225                 230                 235                 240

ccg gac acc tac tgg ccg aag atc cgc gag atc ctc gcc aag tac gac      768
Pro Asp Thr Tyr Trp Pro Lys Ile Arg Glu Ile Leu Ala Lys Tyr Asp
                245                 250                 255

atc ctg ttc atc gcc gac gaa gtg atc tgc ggc ttc ggc cgt acc ggc      816
Ile Leu Phe Ile Ala Asp Glu Val Ile Cys Gly Phe Gly Arg Thr Gly
```

```
                260                 265                 270

gag tgg ttc ggc agc cag tac tac ggc aac gcc ccg gac ctg atg ccg      864
Glu Trp Phe Gly Ser Gln Tyr Tyr Gly Asn Ala Pro Asp Leu Met Pro
        275                 280                 285

atc gcc aag ggc ctc acc tcc ggc tac atc ccc atg ggc ggg gtg gtg      912
Ile Ala Lys Gly Leu Thr Ser Gly Tyr Ile Pro Met Gly Gly Val Val
    290                 295                 300

gtg cgc gac gag atc gtc gaa gtg ctc aac cag ggc ggc gag ttc tac      960
Val Arg Asp Glu Ile Val Glu Val Leu Asn Gln Gly Gly Glu Phe Tyr
305                 310                 315                 320

cac ggc ttc acc tat tcc ggt cac ccg gtg gcg gcc gcc gtg gcc ctg     1008
His Gly Phe Thr Tyr Ser Gly His Pro Val Ala Ala Ala Val Ala Leu
                325                 330                 335

gag aac atc cgc atc ctg cgc gaa gag aag atc atc gag aag gtg aag     1056
Glu Asn Ile Arg Ile Leu Arg Glu Glu Lys Ile Ile Glu Lys Val Lys
            340                 345                 350

gcg gaa acg gca ccg tat ttg cag aaa cgc tgg cag gag ctg gcc gac     1104
Ala Glu Thr Ala Pro Tyr Leu Gln Lys Arg Trp Gln Glu Leu Ala Asp
        355                 360                 365

cac ccg ttg gtg ggc gaa gcg cgc ggg gtc ggc atg gtc gcc gcc ctg     1152
His Pro Leu Val Gly Glu Ala Arg Gly Val Gly Met Val Ala Ala Leu
    370                 375                 380

gag ctg gtc aag aac aag aag acc cgc gag cgt ttc acc gac aag ggc     1200
Glu Leu Val Lys Asn Lys Lys Thr Arg Glu Arg Phe Thr Asp Lys Gly
385                 390                 395                 400

gtc ggg atg ctg tgc cgg gaa cat tgt ttc cgc aac ggt ttg atc atg     1248
Val Gly Met Leu Cys Arg Glu His Cys Phe Arg Asn Gly Leu Ile Met
                405                 410                 415

cgc gcg gtg ggc gac act atg att atc tcg ccg ccg ctg gtg atc gat     1296
Arg Ala Val Gly Asp Thr Met Ile Ile Ser Pro Pro Leu Val Ile Asp
            420                 425                 430

ccg tcg cag atc gat gag ttg atc acc ctg gcg cgc aag tgc ctc gat     1344
Pro Ser Gln Ile Asp Glu Leu Ile Thr Leu Ala Arg Lys Cys Leu Asp
        435                 440                 445

cag acc gcc gcc gcc gtc ctg gct tga                                 1371
Gln Thr Ala Ala Ala Val Leu Ala
    450                 455
```

<210> 8
<211> 456
<212> PRT
<213> 綠膿桿菌(gi 9946143)

<400> 8

Met Asn Ser Gln Ile Thr Asn Ala Lys Thr Arg Glu Trp Gln Ala Leu

1 5 10 15

Ser Arg Asp His His Leu Pro Pro Phe Thr Asp Tyr Lys Gln Leu Asn
20 25 30

Glu Lys Gly Ala Arg Ile Ile Thr Lys Ala Glu Gly Val Tyr Ile Trp
35 40 45

Asp Ser Glu Gly Asn Lys Ile Leu Asp Ala Met Ala Gly Leu Trp Cys
50 55 60

Val Asn Val Gly Tyr Gly Arg Glu Glu Leu Val Gln Ala Ala Thr Arg
65 70 75 80

Gln Met Arg Glu Leu Pro Phe Tyr Asn Leu Phe Phe Gln Thr Ala His
85 90 95

Pro Pro Val Val Glu Leu Ala Lys Ala Ile Ala Asp Val Ala Pro Glu
100 105 110

Gly Met Asn His Val Phe Phe Thr Gly Ser Gly Ser Glu Ala Asn Asp
115 120 125

Thr Val Leu Arg Met Val Arg His Tyr Trp Ala Thr Lys Gly Gln Pro
130 135 140

Gln Lys Lys Val Val Ile Gly Arg Trp Asn Gly Tyr His Gly Ser Thr
145 150 155 160

Val Ala Gly Val Ser Leu Gly Gly Met Lys Ala Leu His Glu Gln Gly
165 170 175

Asp Phe Pro Ile Pro Gly Ile Val His Ile Ala Gln Pro Tyr Trp Tyr
180 185 190

Gly Glu Gly Gly Asp Met Ser Pro Asp Glu Phe Gly Val Trp Ala Ala
195 200 205

Glu Gln Leu Glu Lys Lys Ile Leu Glu Val Gly Glu Glu Asn Val Ala
210 215 220

Ala Phe Ile Ala Glu Pro Ile Gln Gly Ala Gly Gly Val Ile Val Pro
225 230 235 240

Pro Asp Thr Tyr Trp Pro Lys Ile Arg Glu Ile Leu Ala Lys Tyr Asp
245 250 255

Ile Leu Phe Ile Ala Asp Glu Val Ile Cys Gly Phe Gly Arg Thr Gly
260 265 270

Glu Trp Phe Gly Ser Gln Tyr Tyr Gly Asn Ala Pro Asp Leu Met Pro
275 280 285

Ile Ala Lys Gly Leu Thr Ser Gly Tyr Ile Pro Met Gly Gly Val Val
290 295 300

Val Arg Asp Glu Ile Val Glu Val Leu Asn Gln Gly Gly Glu Phe Tyr
305 310 315 320

His Gly Phe Thr Tyr Ser Gly His Pro Val Ala Ala Ala Val Ala Leu
325 330 335

Glu Asn Ile Arg Ile Leu Arg Glu Glu Lys Ile Ile Glu Lys Val Lys
340 345 350

Ala Glu Thr Ala Pro Tyr Leu Gln Lys Arg Trp Gln Glu Leu Ala Asp
355 360 365

His Pro Leu Val Gly Glu Ala Arg Gly Val Gly Met Val Ala Ala Leu
370 375 380

Glu Leu Val Lys Asn Lys Lys Thr Arg Glu Arg Phe Thr Asp Lys Gly
385 390 395 400

Val Gly Met Leu Cys Arg Glu His Cys Phe Arg Asn Gly Leu Ile Met
405 410 415

Arg Ala Val Gly Asp Thr Met Ile Ile Ser Pro Pro Leu Val Ile Asp
420 425 430

Pro Ser Gln Ile Asp Glu Leu Ile Thr Leu Ala Arg Lys Cys Leu Asp
435 440 445

Gln Thr Ala Ala Ala Val Leu Ala
450 455

<210> 9
<211> 70
<212> DNA

<213> 人造

<220>
<223> 引子

<400> 9
ggggacaagt ttgtacaaaa aagcaggcta ggaggaatta accatgaaca gccaaatcac 60

caacgccaag 70

<210> 10
<211> 49
<212> DNA
<213> 人造

<220>
<223> 引子

<400> 10
ggggaccact ttgtacaaga aagctgggtt caagccagga cggcggcgg 49

<210> 11
<211> 1365
<212> DNA
<213> 環狀假單胞菌

<220>
<221> CDS
<222> (1)..(1365)

<400> 11
atg agt gcc aac aac ccg caa acc ctc gaa tgg cag gcc ctg agc agc 48
Met Ser Ala Asn Asn Pro Gln Thr Leu Glu Trp Gln Ala Leu Ser Ser
1 5 10 15

gag cat cac ctg gca ccg ttc agc gac tac aaa caa ctg aaa gag aaa 96
Glu His His Leu Ala Pro Phe Ser Asp Tyr Lys Gln Leu Lys Glu Lys
20 25 30

ggc ccg cgc atc atc acc cgt gcc gag ggc gtt tat ctg tgg gac agc 144
Gly Pro Arg Ile Ile Thr Arg Ala Glu Gly Val Tyr Leu Trp Asp Ser
35 40 45

gag ggc aac aag atc ctc gat ggc atg tcc ggc ctg tgg tgc gtg gcc 192
Glu Gly Asn Lys Ile Leu Asp Gly Met Ser Gly Leu Trp Cys Val Ala
50 55 60

atc ggt tat ggc cgc gaa gaa ctg gcc gac gca gcc agc aaa cag atg 240
Ile Gly Tyr Gly Arg Glu Glu Leu Ala Asp Ala Ala Ser Lys Gln Met
65 70 75 80

cgc gag ctg ccg tac tac aac ctg ttc ttc cag acc gcc cac ccg ccg 288
Arg Glu Leu Pro Tyr Tyr Asn Leu Phe Phe Gln Thr Ala His Pro Pro
85 90 95

gtg ctg gaa ctg gcc aag gcc atc tcc gac atc gct ccc gag ggc atg 336

```
Val Leu Glu Leu Ala Lys Ala Ile Ser Asp Ile Ala Pro Glu Gly Met
            100                 105                 110

aac cat gtg ttc ttc acc ggt tca ggc tct gaa ggc aat gac acg atg      384
Asn His Val Phe Phe Thr Gly Ser Gly Ser Glu Gly Asn Asp Thr Met
        115                 120                 125

ctg cgc atg gtt cgt cat tac tgg gcg ctg aaa ggc cag ccg aac aag      432
Leu Arg Met Val Arg His Tyr Trp Ala Leu Lys Gly Gln Pro Asn Lys
    130                 135                 140

aaa acc atc atc agc cgc gtc aat ggc tac cac ggc tcc acc gtc gcc      480
Lys Thr Ile Ile Ser Arg Val Asn Gly Tyr His Gly Ser Thr Val Ala
145                 150                 155                 160

ggt gcc agc ctg ggt ggc atg acc tac atg cac gaa cag ggc gac ctg      528
Gly Ala Ser Leu Gly Gly Met Thr Tyr Met His Glu Gln Gly Asp Leu
                165                 170                 175

ccg atc ccg ggg gtg gtg cac att cca cag cct tac tgg ttc ggc gaa      576
Pro Ile Pro Gly Val Val His Ile Pro Gln Pro Tyr Trp Phe Gly Glu
            180                 185                 190

ggc ggc gac atg acg ccg gac gag ttc ggc atc tgg gcg gcc gag caa      624
Gly Gly Asp Met Thr Pro Asp Glu Phe Gly Ile Trp Ala Ala Glu Gln
        195                 200                 205

ctg gaa aag aaa att ctc gag ctg ggc gtc gag aac gtc ggt gcg ttc      672
Leu Glu Lys Lys Ile Leu Glu Leu Gly Val Glu Asn Val Gly Ala Phe
    210                 215                 220

att gcc gag cca atc cag ggc gcg ggc ggt gtg att gtc ccg cct gat      720
Ile Ala Glu Pro Ile Gln Gly Ala Gly Gly Val Ile Val Pro Pro Asp
225                 230                 235                 240

tcc tac tgg ccg aag atc aag gaa atc ctt tcc cgc tac gac atc ctg      768
Ser Tyr Trp Pro Lys Ile Lys Glu Ile Leu Ser Arg Tyr Asp Ile Leu
                245                 250                 255

ttc gcc gcc gat gag gtg att tgt ggc ttc ggg cgt acc agt gag tgg      816
Phe Ala Ala Asp Glu Val Ile Cys Gly Phe Gly Arg Thr Ser Glu Trp
            260                 265                 270

ttc ggt agc gat ttc tat ggc ctc agg ccg gac atg atg acc atc gcc      864
Phe Gly Ser Asp Phe Tyr Gly Leu Arg Pro Asp Met Met Thr Ile Ala
        275                 280                 285

aaa ggc ctg acc tcc ggt tac gta ccg atg ggc ggc ctg atc gtg cgc      912
Lys Gly Leu Thr Ser Gly Tyr Val Pro Met Gly Gly Leu Ile Val Arg
    290                 295                 300

gat gaa atc gtt gcg gtg ctc aat gag ggt ggc gat ttc aat cac ggc      960
Asp Glu Ile Val Ala Val Leu Asn Glu Gly Gly Asp Phe Asn His Gly
305                 310                 315                 320

ttt acc tac tcc ggg cac ccg gtg gcg gcc gcg gtt gcg ctg gag aac     1008
Phe Thr Tyr Ser Gly His Pro Val Ala Ala Ala Val Ala Leu Glu Asn
                325                 330                 335
```

```
atc cgt atc ctg cgc gaa gaa aag atc gtc gaa cgg gtc agg tcg gaa      1056
Ile Arg Ile Leu Arg Glu Glu Lys Ile Val Glu Arg Val Arg Ser Glu
            340                 345                 350

acg gca ccg tat ttg caa aag cgt ttg cgt gag ttg agc gat cat ccg      1104
Thr Ala Pro Tyr Leu Gln Lys Arg Leu Arg Glu Leu Ser Asp His Pro
        355                 360                 365

ctg gtg ggc gaa gtc cgg ggt gtc ggg ctg ctc ggg gcc att gag ctg      1152
Leu Val Gly Glu Val Arg Gly Val Gly Leu Leu Gly Ala Ile Glu Leu
    370                 375                 380

gtg aag gac aag acc acc cgc gag cgc tat acc gac aag ggc gcg gga      1200
Val Lys Asp Lys Thr Thr Arg Glu Arg Tyr Thr Asp Lys Gly Ala Gly
385                 390                 395                 400

atg atc tgt cga acc ttc tgc ttc gac aat ggc ctg atc atg cgg gct      1248
Met Ile Cys Arg Thr Phe Cys Phe Asp Asn Gly Leu Ile Met Arg Ala
                405                 410                 415

gtg ggc gat acc atg atc att gcg ccg cca ctg gtg atc agt ttt gcg      1296
Val Gly Asp Thr Met Ile Ile Ala Pro Pro Leu Val Ile Ser Phe Ala
            420                 425                 430

caa atc gat gag ctg gta gag aag gcg cgc acg tgt ctg gat ctg acg      1344
Gln Ile Asp Glu Leu Val Glu Lys Ala Arg Thr Cys Leu Asp Leu Thr
        435                 440                 445

ctg gcg gtg ttg cag ggc tga                                         1365
Leu Ala Val Leu Gln Gly
    450
```

<210> 12
<211> 454
<212> PRT
<213> 環狀假單胞菌

<400> 12

```
Met Ser Ala Asn Asn Pro Gln Thr Leu Glu Trp Gln Ala Leu Ser Ser
1               5                   10                  15

Glu His His Leu Ala Pro Phe Ser Asp Tyr Lys Gln Leu Lys Glu Lys
            20                  25                  30

Gly Pro Arg Ile Ile Thr Arg Ala Glu Gly Val Tyr Leu Trp Asp Ser
        35                  40                  45

Glu Gly Asn Lys Ile Leu Asp Gly Met Ser Gly Leu Trp Cys Val Ala
    50                  55                  60

Ile Gly Tyr Gly Arg Glu Glu Leu Ala Asp Ala Ala Ser Lys Gln Met
65                  70                  75                  80
```

Arg Glu Leu Pro Tyr Tyr Asn Leu Phe Phe Gln Thr Ala His Pro Pro
85 90 95

Val Leu Glu Leu Ala Lys Ala Ile Ser Asp Ile Ala Pro Glu Gly Met
100 105 110

Asn His Val Phe Phe Thr Gly Ser Gly Ser Glu Gly Asn Asp Thr Met
115 120 125

Leu Arg Met Val Arg His Tyr Trp Ala Leu Lys Gly Gln Pro Asn Lys
130 135 140

Lys Thr Ile Ile Ser Arg Val Asn Gly Tyr His Gly Ser Thr Val Ala
145 150 155 160

Gly Ala Ser Leu Gly Gly Met Thr Tyr Met His Glu Gln Gly Asp Leu
165 170 175

Pro Ile Pro Gly Val Val His Ile Pro Gln Pro Tyr Trp Phe Gly Glu
180 185 190

Gly Gly Asp Met Thr Pro Asp Glu Phe Gly Ile Trp Ala Ala Glu Gln
195 200 205

Leu Glu Lys Lys Ile Leu Glu Leu Gly Val Glu Asn Val Gly Ala Phe
210 215 220

Ile Ala Glu Pro Ile Gln Gly Ala Gly Gly Val Ile Val Pro Pro Asp
225 230 235 240

Ser Tyr Trp Pro Lys Ile Lys Glu Ile Leu Ser Arg Tyr Asp Ile Leu
245 250 255

Phe Ala Ala Asp Glu Val Ile Cys Gly Phe Gly Arg Thr Ser Glu Trp
260 265 270

Phe Gly Ser Asp Phe Tyr Gly Leu Arg Pro Asp Met Met Thr Ile Ala
275 280 285

Lys Gly Leu Thr Ser Gly Tyr Val Pro Met Gly Gly Leu Ile Val Arg
290 295 300

Asp Glu Ile Val Ala Val Leu Asn Glu Gly Gly Asp Phe Asn His Gly
305 310 315 320

Phe Thr Tyr Ser Gly His Pro Val Ala Ala Ala Val Ala Leu Glu Asn
325 330 335

Ile Arg Ile Leu Arg Glu Glu Lys Ile Val Glu Arg Val Arg Ser Glu
340 345 350

Thr Ala Pro Tyr Leu Gln Lys Arg Leu Arg Glu Leu Ser Asp His Pro
355 360 365

Leu Val Gly Glu Val Arg Gly Val Gly Leu Leu Gly Ala Ile Glu Leu
370 375 380

Val Lys Asp Lys Thr Thr Arg Glu Arg Tyr Thr Asp Lys Gly Ala Gly
385 390 395 400

Met Ile Cys Arg Thr Phe Cys Phe Asp Asn Gly Leu Ile Met Arg Ala
405 410 415

Val Gly Asp Thr Met Ile Ile Ala Pro Pro Leu Val Ile Ser Phe Ala
420 425 430

Gln Ile Asp Glu Leu Val Glu Lys Ala Arg Thr Cys Leu Asp Leu Thr
435 440 445

Leu Ala Val Leu Gln Gly
450

<210> 13
<211> 1365
<212> DNA
<213> 人造

<220>
<223> 環狀假單胞菌密碼子最佳化轉胺苷基因

<400> 13
atgtctgcta acaatccaca aactctggaa tggcaggcac tgagctccga acatcacctg 60

gctccgttct ccgactacaa acaactgaaa gagaaaggcc cgcgtatcat tacccgcgct 120

gaaggtgtgt acctgtggga ttctgaaggc aacaaaattc tggacggtat gagcggcctg 180

tggtgcgtag caatcggtta tggccgtgaa gaactggctg acgcggcgag caaacagatg 240

cgtgaactgc cgtattataa cctgttcttc caaaccgcac acccgccggt tctggaactg 300

gctaaagcta tcagcgatat cgcaccggag ggcatgaatc acgtcttctt cactggttcc 360

ggtagcgaag gcaacgacac gatgctgcgc atggtacgtc actattgggc gctgaagggc 420

cagccgaaca agaaaacgat tatcagccgt gtaaacggtt atcacggcag caccgttgcg 480

ggtgcgagcc tgggcggtat gacctacatg cacgaacagg gtgacctgcc gatcccgggt 540

gtagtgcaca ttccgcagcc gtattggttc ggtgaaggcg gtgacatgac gccggacgaa 600

ttcggcatct gggcggcaga gcagctggaa aagaaaatcc tggaactggg cgtggaaaac 660

gtcggcgcgt tcatcgcgga accgattcag ggcgcgggcg gcgtaattgt tccgccggac 720

agctactggc caaaaatcaa agagatcctg tctcgttacg acatcctgtt cgccgcagac 780

gaagtgatct gcggttttgg ccgcacctct gaatggttcg gctccgactt ctacggtctg 840

cgtccggaca tgatgaccat cgccaaaggc ctgacctccg gttatgttcc tatgggtggc 900

ctgatcgtgc gcgacgaaat tgttgcggtt ctgaacgaag gcggcgattt caaccacggc 960

ttcacctatt ccggtcaccc agttgctgct gctgtagcac tggaaaacat ccgcatcctg 1020

cgtgaagaaa agatcgtaga acgcgtacgt tccgaaaccg caccttacct gcagaagcgc 1080

ctgcgcgaac tgagcgacca ccctctggta ggtgaagttc gcggcgtggg cctgctgggc 1140

gcgatcgagc tggtgaaaga caaaactacc cgtgaacgtt acaccgacaa aggcgcaggc 1200

atgatctgcc gtacctttg cttcgataac ggtctgatca tgcgcgcagt cggtgatacc 1260

atgatcattg ctccgcctct ggttatttct tttgcccaga ttgatgagct ggtcgaaaaa 1320

gcgcgcactt gtctggatct gactctggct gttctgcagg gttaa 1365

<210> 14
<211> 849
<212> DNA
<213> 枯草桿菌(gi 16078032)

<220>
<221> CDS
<222> (1)..(849)

<400> 14

atg aag gtt tta gtc aat ggc cgg ctg att ggg cgc agt gaa gca tca 48
Met Lys Val Leu Val Asn Gly Arg Leu Ile Gly Arg Ser Glu Ala Ser
1 5 10 15

atc gat ttg gaa gat cgc ggt tat cag ttt ggt gac ggc atc tat gaa 96
Ile Asp Leu Glu Asp Arg Gly Tyr Gln Phe Gly Asp Gly Ile Tyr Glu
20 25 30

gtg atc agg gtg tac aaa gga gta ttg ttc ggc tta cgt gag cat gca 144
Val Ile Arg Val Tyr Lys Gly Val Leu Phe Gly Leu Arg Glu His Ala
35 40 45

gag cgt ttt ttc aga agt gct gct gaa atc gga att tca ctg cca ttc 192

```
Glu Arg Phe Phe Arg Ser Ala Ala Glu Ile Gly Ile Ser Leu Pro Phe
    50                  55                  60

agt ata gaa gat ctc gag tgg gac ctg caa aag ctt gta cag gaa aat      240
Ser Ile Glu Asp Leu Glu Trp Asp Leu Gln Lys Leu Val Gln Glu Asn
65                  70                  75                  80

gcg gtc agt gag gga gcg gta tac att cag aca aca aga ggt gtg gcc      288
Ala Val Ser Glu Gly Ala Val Tyr Ile Gln Thr Thr Arg Gly Val Ala
                85                  90                  95

ccg cga aaa cac cag tat gaa gcc ggc ctc gag ccg cag act act gcc      336
Pro Arg Lys His Gln Tyr Glu Ala Gly Leu Glu Pro Gln Thr Thr Ala
            100                 105                 110

tat acg ttt acg gtg aaa aaa ccg gag caa gag cag gca tac gga gtg      384
Tyr Thr Phe Thr Val Lys Lys Pro Glu Gln Glu Gln Ala Tyr Gly Val
        115                 120                 125

gcg gcc att aca gat gag gat ctt cgc tgg tta aga tgt gat atc aaa      432
Ala Ala Ile Thr Asp Glu Asp Leu Arg Trp Leu Arg Cys Asp Ile Lys
    130                 135                 140

agt ctg aat tta ctg tat aat gtc atg acg aag caa agg gcc tat gaa      480
Ser Leu Asn Leu Leu Tyr Asn Val Met Thr Lys Gln Arg Ala Tyr Glu
145                 150                 155                 160

gcc gga gca ttt gaa gcc att tta ctt agg gac ggc gtt gtt acg gag      528
Ala Gly Ala Phe Glu Ala Ile Leu Leu Arg Asp Gly Val Val Thr Glu
                165                 170                 175

ggt aca tcc tct aac gtt tat gcc gtt atc aac ggc aca gtg cga aca      576
Gly Thr Ser Ser Asn Val Tyr Ala Val Ile Asn Gly Thr Val Arg Thr
            180                 185                 190

cat ccg gct aat cgg ctc att ctc aat gga att aca cgg atg aat att      624
His Pro Ala Asn Arg Leu Ile Leu Asn Gly Ile Thr Arg Met Asn Ile
        195                 200                 205

tta gga ctg att gag aag aat ggg atc aaa ctg gat gag act cct gtc      672
Leu Gly Leu Ile Glu Lys Asn Gly Ile Lys Leu Asp Glu Thr Pro Val
    210                 215                 220

agt gaa gaa gag ttg aaa cag gcg gaa gag atc ttt att tcg tca acg      720
Ser Glu Glu Glu Leu Lys Gln Ala Glu Glu Ile Phe Ile Ser Ser Thr
225                 230                 235                 240

acg gca gaa att att ccg gtc gtg acg ctc gat gga caa tcg atc gga      768
Thr Ala Glu Ile Ile Pro Val Val Thr Leu Asp Gly Gln Ser Ile Gly
                245                 250                 255

agc ggg aaa ccc gga ccg gtg acc aaa cag ctt cag gct gct ttt caa      816
Ser Gly Lys Pro Gly Pro Val Thr Lys Gln Leu Gln Ala Ala Phe Gln
            260                 265                 270

gaa agc att caa cag gct gct agc att tca taa                         849
Glu Ser Ile Gln Gln Ala Ala Ser Ile Ser
        275                 280
```

<210> 15
<211> 282
<212> PRT
<213> 枯草桿菌(gi 16078032)

<400> 15

Met Lys Val Leu Val Asn Gly Arg Leu Ile Gly Arg Ser Glu Ala Ser
1 5 10 15

Ile Asp Leu Glu Asp Arg Gly Tyr Gln Phe Gly Asp Gly Ile Tyr Glu
20 25 30

Val Ile Arg Val Tyr Lys Gly Val Leu Phe Gly Leu Arg Glu His Ala
35 40 45

Glu Arg Phe Phe Arg Ser Ala Ala Glu Ile Gly Ile Ser Leu Pro Phe
50 55 60

Ser Ile Glu Asp Leu Glu Trp Asp Leu Gln Lys Leu Val Gln Glu Asn
65 70 75 80

Ala Val Ser Glu Gly Ala Val Tyr Ile Gln Thr Thr Arg Gly Val Ala
85 90 95

Pro Arg Lys His Gln Tyr Glu Ala Gly Leu Glu Pro Gln Thr Thr Ala
100 105 110

Tyr Thr Phe Thr Val Lys Lys Pro Glu Gln Glu Gln Ala Tyr Gly Val
115 120 125

Ala Ala Ile Thr Asp Glu Asp Leu Arg Trp Leu Arg Cys Asp Ile Lys
130 135 140

Ser Leu Asn Leu Leu Tyr Asn Val Met Thr Lys Gln Arg Ala Tyr Glu
145 150 155 160

Ala Gly Ala Phe Glu Ala Ile Leu Leu Arg Asp Gly Val Val Thr Glu
165 170 175

Gly Thr Ser Ser Asn Val Tyr Ala Val Ile Asn Gly Thr Val Arg Thr
180 185 190

His Pro Ala Asn Arg Leu Ile Leu Asn Gly Ile Thr Arg Met Asn Ile
195 200 205

```
Leu Gly Leu Ile Glu Lys Asn Gly Ile Lys Leu Asp Glu Thr Pro Val
    210                 215                 220

Ser Glu Glu Glu Leu Lys Gln Ala Glu Glu Ile Phe Ile Ser Ser Thr
225                 230                 235                 240

Thr Ala Glu Ile Ile Pro Val Val Thr Leu Asp Gly Gln Ser Ile Gly
                245                 250                 255

Ser Gly Lys Pro Gly Pro Val Thr Lys Gln Leu Gln Ala Ala Phe Gln
            260                 265                 270

Glu Ser Ile Gln Gln Ala Ala Ser Ile Ser
        275                 280
```

<210> 16
<211> 1347
<212> DNA
<213> 枯草桿菌(gi 16080075)

<220>
<221> CDS
<222> (1)..(1347)

<400> 16

```
atg act cat gat ttg ata gaa aaa agt aaa aag cac ctc tgg ctg cca      48
Met Thr His Asp Leu Ile Glu Lys Ser Lys Lys His Leu Trp Leu Pro
1               5                   10                  15

ttt acc caa atg aaa gat tat gat gaa aac ccc tta atc atc gaa agc      96
Phe Thr Gln Met Lys Asp Tyr Asp Glu Asn Pro Leu Ile Ile Glu Ser
            20                  25                  30

ggg act gga atc aaa gtc aaa gac ata aac ggc aag gaa tac tat gac     144
Gly Thr Gly Ile Lys Val Lys Asp Ile Asn Gly Lys Glu Tyr Tyr Asp
        35                  40                  45

ggt ttt tca tcg gtt tgg ctt aat gtc cac gga cac cgc aaa aaa gaa     192
Gly Phe Ser Ser Val Trp Leu Asn Val His Gly His Arg Lys Lys Glu
    50                  55                  60

cta gat gac gcc ata aaa aaa cag ctc gga aaa att gcg cac tcc acg     240
Leu Asp Asp Ala Ile Lys Lys Gln Leu Gly Lys Ile Ala His Ser Thr
65                  70                  75                  80

tta ttg ggc atg acc aat gtt cca gca acc cag ctt gcc gaa aca tta     288
Leu Leu Gly Met Thr Asn Val Pro Ala Thr Gln Leu Ala Glu Thr Leu
                85                  90                  95

atc gac atc agc cca aaa aag ctc acg cgg gtc ttt tat tca gac agc     336
Ile Asp Ile Ser Pro Lys Lys Leu Thr Arg Val Phe Tyr Ser Asp Ser
            100                 105                 110
```

```
ggc gca gag gcg atg gaa ata gcc cta aaa atg gcg ttt cag tat tgg      384
Gly Ala Glu Ala Met Glu Ile Ala Leu Lys Met Ala Phe Gln Tyr Trp
        115                 120                 125

aag aac atc ggg aag ccc gag aaa caa aaa ttc atc gca atg aaa aac      432
Lys Asn Ile Gly Lys Pro Glu Lys Gln Lys Phe Ile Ala Met Lys Asn
    130                 135                 140

ggg tat cac ggt gat acg att ggc gcc gtc agt gtc ggt tca att gag      480
Gly Tyr His Gly Asp Thr Ile Gly Ala Val Ser Val Gly Ser Ile Glu
145                 150                 155                 160

ctt ttt cac cac gta tac ggc ccg ttg atg ttc gag agt tac aag gcc      528
Leu Phe His His Val Tyr Gly Pro Leu Met Phe Glu Ser Tyr Lys Ala
                165                 170                 175

ccg att cct tat gtg tat cgt tct gaa agc ggt gat cct gat gag tgc      576
Pro Ile Pro Tyr Val Tyr Arg Ser Glu Ser Gly Asp Pro Asp Glu Cys
            180                 185                 190

cgt gat cag tgc ctc cga gag ctt gca cag ctg ctt gag gaa cat cat      624
Arg Asp Gln Cys Leu Arg Glu Leu Ala Gln Leu Leu Glu Glu His His
        195                 200                 205

gag gaa att gcc gcg ctt tcc att gaa tca atg gta caa ggc gcg tcc      672
Glu Glu Ile Ala Ala Leu Ser Ile Glu Ser Met Val Gln Gly Ala Ser
    210                 215                 220

ggt atg atc gtg atg ccg gaa gga tat ttg gca ggc gtg cgc gag cta      720
Gly Met Ile Val Met Pro Glu Gly Tyr Leu Ala Gly Val Arg Glu Leu
225                 230                 235                 240

tgt aca aca tac gat gtc tta atg atc gtt gat gaa gtc gct aca ggc      768
Cys Thr Thr Tyr Asp Val Leu Met Ile Val Asp Glu Val Ala Thr Gly
                245                 250                 255

ttt ggc cgt aca gga aaa atg ttt gcg tgc gag cac gag aat gtc cag      816
Phe Gly Arg Thr Gly Lys Met Phe Ala Cys Glu His Glu Asn Val Gln
            260                 265                 270

cct gat ctg atg gct gcc ggt aaa ggc att aca gga ggc tat ttg cca      864
Pro Asp Leu Met Ala Ala Gly Lys Gly Ile Thr Gly Gly Tyr Leu Pro
        275                 280                 285

att gcc gtt acg ttt gcc act gaa gac atc tat aag gca ttc tat gat      912
Ile Ala Val Thr Phe Ala Thr Glu Asp Ile Tyr Lys Ala Phe Tyr Asp
    290                 295                 300

gat tat gaa aac cta aaa acc ttt ttc cat ggc cat tcc tat aca ggc      960
Asp Tyr Glu Asn Leu Lys Thr Phe Phe His Gly His Ser Tyr Thr Gly
305                 310                 315                 320

aat cag ctt ggc tgt gcg gtt gcg ctt gaa aat ctg gca tta ttt gaa     1008
Asn Gln Leu Gly Cys Ala Val Ala Leu Glu Asn Leu Ala Leu Phe Glu
                325                 330                 335

tct gaa aac att gtg gaa caa gta gcg gaa aaa agt aaa aag ctc cat     1056
Ser Glu Asn Ile Val Glu Gln Val Ala Glu Lys Ser Lys Lys Leu His
```

```
            340                 345                 350

ttt ctt ctt caa gat ctg cac gct ctt cct cat gtt ggg gat att cgg    1104
Phe Leu Leu Gln Asp Leu His Ala Leu Pro His Val Gly Asp Ile Arg
        355                 360                 365

cag ctt ggc ttt atg tgc ggt gca gag ctt gta cga tca aag gaa act    1152
Gln Leu Gly Phe Met Cys Gly Ala Glu Leu Val Arg Ser Lys Glu Thr
    370                 375                 380

aaa gaa cct tac ccg gct gat cgg cgg att gga tac aaa gtt tcc tta    1200
Lys Glu Pro Tyr Pro Ala Asp Arg Arg Ile Gly Tyr Lys Val Ser Leu
385                 390                 395                 400

aaa atg aga gag tta gga atg ctg aca aga ccg ctt ggg gac gtg att    1248
Lys Met Arg Glu Leu Gly Met Leu Thr Arg Pro Leu Gly Asp Val Ile
                405                 410                 415

gca ttt ctt cct cct ctt gcc agc aca gct gaa gag ctc tcg gaa atg    1296
Ala Phe Leu Pro Pro Leu Ala Ser Thr Ala Glu Glu Leu Ser Glu Met
            420                 425                 430

gtt gcc att atg aaa caa gcg atc cac gag gtt acg agc ctt gaa gat    1344
Val Ala Ile Met Lys Gln Ala Ile His Glu Val Thr Ser Leu Glu Asp
        435                 440                 445

tga                                                                1347
```

<210> 17
<211> 448
<212> PRT
<213> 枯草桿菌(gi 16080075)

<400> 17

```
Met Thr His Asp Leu Ile Glu Lys Ser Lys Lys His Leu Trp Leu Pro
1               5                   10                  15

Phe Thr Gln Met Lys Asp Tyr Asp Glu Asn Pro Leu Ile Ile Glu Ser
            20                  25                  30

Gly Thr Gly Ile Lys Val Lys Asp Ile Asn Gly Lys Glu Tyr Tyr Asp
        35                  40                  45

Gly Phe Ser Ser Val Trp Leu Asn Val His Gly His Arg Lys Lys Glu
    50                  55                  60

Leu Asp Asp Ala Ile Lys Lys Gln Leu Gly Lys Ile Ala His Ser Thr
65                  70                  75                  80

Leu Leu Gly Met Thr Asn Val Pro Ala Thr Gln Leu Ala Glu Thr Leu
                85                  90                  95
```

Ile Asp Ile Ser Pro Lys Lys Leu Thr Arg Val Phe Tyr Ser Asp Ser
100 105 110

Gly Ala Glu Ala Met Glu Ile Ala Leu Lys Met Ala Phe Gln Tyr Trp
115 120 125

Lys Asn Ile Gly Lys Pro Glu Lys Gln Lys Phe Ile Ala Met Lys Asn
130 135 140

Gly Tyr His Gly Asp Thr Ile Gly Ala Val Ser Val Gly Ser Ile Glu
145 150 155 160

Leu Phe His His Val Tyr Gly Pro Leu Met Phe Glu Ser Tyr Lys Ala
165 170 175

Pro Ile Pro Tyr Val Tyr Arg Ser Glu Ser Gly Asp Pro Asp Glu Cys
180 185 190

Arg Asp Gln Cys Leu Arg Glu Leu Ala Gln Leu Leu Glu Glu His His
195 200 205

Glu Glu Ile Ala Ala Leu Ser Ile Glu Ser Met Val Gln Gly Ala Ser
210 215 220

Gly Met Ile Val Met Pro Glu Gly Tyr Leu Ala Gly Val Arg Glu Leu
225 230 235 240

Cys Thr Thr Tyr Asp Val Leu Met Ile Val Asp Glu Val Ala Thr Gly
245 250 255

Phe Gly Arg Thr Gly Lys Met Phe Ala Cys Glu His Glu Asn Val Gln
260 265 270

Pro Asp Leu Met Ala Ala Gly Lys Gly Ile Thr Gly Gly Tyr Leu Pro
275 280 285

Ile Ala Val Thr Phe Ala Thr Glu Asp Ile Tyr Lys Ala Phe Tyr Asp
290 295 300

Asp Tyr Glu Asn Leu Lys Thr Phe Phe His Gly His Ser Tyr Thr Gly
305 310 315 320

Asn Gln Leu Gly Cys Ala Val Ala Leu Glu Asn Leu Ala Leu Phe Glu
325 330 335

Ser Glu Asn Ile Val Glu Gln Val Ala Glu Lys Ser Lys Lys Leu His
340 345 350

Phe Leu Leu Gln Asp Leu His Ala Leu Pro His Val Gly Asp Ile Arg
355 360 365

Gln Leu Gly Phe Met Cys Gly Ala Glu Leu Val Arg Ser Lys Glu Thr
370 375 380

Lys Glu Pro Tyr Pro Ala Asp Arg Arg Ile Gly Tyr Lys Val Ser Leu
385 390 395 400

Lys Met Arg Glu Leu Gly Met Leu Thr Arg Pro Leu Gly Asp Val Ile
405 410 415

Ala Phe Leu Pro Pro Leu Ala Ser Thr Ala Glu Glu Leu Ser Glu Met
420 425 430

Val Ala Ile Met Lys Gln Ala Ile His Glu Val Thr Ser Leu Glu Asp
435 440 445

<210> 18
<211> 1467
<212> DNA
<213> 球狀紅桿菌

<220>
<221> CDS
<222> (1)..(1467)

<400> 18

```
atg ccc ggt tgc ggg ggc ttg ccc ggg aat gaa ccg aaa tgc gga cga      48
Met Pro Gly Cys Gly Gly Leu Pro Gly Asn Glu Pro Lys Cys Gly Arg
1               5                  10                  15

gag ggg agg tcg gcg atg acg cgg aat gac gcg acg aat gct gcc gga      96
Glu Gly Arg Ser Ala Met Thr Arg Asn Asp Ala Thr Asn Ala Ala Gly
            20                  25                  30

gcg gtg ggc gcg gcg atg cgg gat cac atc ctc ttg cct gca cag gaa     144
Ala Val Gly Ala Ala Met Arg Asp His Ile Leu Leu Pro Ala Gln Glu
        35                  40                  45

atg gcg aag ctc ggc aag tcc gcg cag ccg gtg ctg act cat gcc gag     192
Met Ala Lys Leu Gly Lys Ser Ala Gln Pro Val Leu Thr His Ala Glu
    50                  55                  60

ggc atc tat gtc cat acc gag gac ggc cgc cgc ctg atc gac ggg ccg     240
Gly Ile Tyr Val His Thr Glu Asp Gly Arg Arg Leu Ile Asp Gly Pro
```

```
65                  70                  75                  80

gcg ggc atg tgg tgc gcg cag gtg ggc tac ggc cgc cgc gag atc gtc      288
Ala Gly Met Trp Cys Ala Gln Val Gly Tyr Gly Arg Arg Glu Ile Val
                85                  90                  95

gat gcc atg gcg cat cag gcg atg gtg ctg ccc tat gcc tcg ccc tgg      336
Asp Ala Met Ala His Gln Ala Met Val Leu Pro Tyr Ala Ser Pro Trp
            100                 105                 110

tat atg gcc acg agc ccc gcg gcg cgg ctg gcg gag aag atc gcc acg      384
Tyr Met Ala Thr Ser Pro Ala Ala Arg Leu Ala Glu Lys Ile Ala Thr
        115                 120                 125

ctg acg ccg ggc gat ctc aac cgg atc ttt ttc acc acg ggc ggg tcg      432
Leu Thr Pro Gly Asp Leu Asn Arg Ile Phe Phe Thr Thr Gly Gly Ser
    130                 135                 140

acc gcg gtg gac agc gcg ctg cgc ttc tcg gaa ttc tac aac aac gtg      480
Thr Ala Val Asp Ser Ala Leu Arg Phe Ser Glu Phe Tyr Asn Asn Val
145                 150                 155                 160

ctg ggc cgg ccg cag aag aag cgc atc atc gtg cgc tac gac ggc tat      528
Leu Gly Arg Pro Gln Lys Lys Arg Ile Ile Val Arg Tyr Asp Gly Tyr
                165                 170                 175

cac ggc tcg acg gcg ctc acc gcc gcc tgc acc ggc cgc acc ggc aac      576
His Gly Ser Thr Ala Leu Thr Ala Ala Cys Thr Gly Arg Thr Gly Asn
            180                 185                 190

tgg ccg aac ttc gac atc gcg cag gac cgg atc tcg ttc ctc tcg agc      624
Trp Pro Asn Phe Asp Ile Ala Gln Asp Arg Ile Ser Phe Leu Ser Ser
        195                 200                 205

ccc aat ccg cgc cac gcc ggc aac cgc agc cag gag gcg ttc ctc gac      672
Pro Asn Pro Arg His Ala Gly Asn Arg Ser Gln Glu Ala Phe Leu Asp
    210                 215                 220

gat ctg gtg cag gaa ttc gag gac cgg atc gag agc ctc ggc ccc gac      720
Asp Leu Val Gln Glu Phe Glu Asp Arg Ile Glu Ser Leu Gly Pro Asp
225                 230                 235                 240

acg atc gcg gcc ttc ctg gcc gag ccg atc ctc gcc tcg ggc ggc gtc      768
Thr Ile Ala Ala Phe Leu Ala Glu Pro Ile Leu Ala Ser Gly Gly Val
                245                 250                 255

att att ccg ccc gca ggc tat cat gcg cgc ttc aag gcg atc tgc gag      816
Ile Ile Pro Pro Ala Gly Tyr His Ala Arg Phe Lys Ala Ile Cys Glu
            260                 265                 270

aag cac gac atc ctc tat atc tcg gac gag gtg gtg acg ggc ttc ggc      864
Lys His Asp Ile Leu Tyr Ile Ser Asp Glu Val Val Thr Gly Phe Gly
        275                 280                 285

cgt tgc ggc gag tgg ttc gcc tcg gag aag gtg ttc ggg gtg gtg ccg      912
Arg Cys Gly Glu Trp Phe Ala Ser Glu Lys Val Phe Gly Val Val Pro
    290                 295                 300

gac atc atc acc ttc gcc aag ggc gtg acc tcg ggc tat gtg ccg ctc      960
```

```
Asp Ile Ile Thr Phe Ala Lys Gly Val Thr Ser Gly Tyr Val Pro Leu
305                 310                 315                 320

ggc ggc ctt gcg atc tcc gag gcg gtg ctg gcg cgg atc tcg ggc gag  1008
Gly Gly Leu Ala Ile Ser Glu Ala Val Leu Ala Arg Ile Ser Gly Glu
                325                 330                 335

aat gcc aag gga agc tgg ttc acc aac ggc tat acc tac agc aat cag  1056
Asn Ala Lys Gly Ser Trp Phe Thr Asn Gly Tyr Thr Tyr Ser Asn Gln
            340                 345                 350

ccg gtg gcc tgc gcc gcg gcg ctt gcc aac atc gag ctg atg gag cgc  1104
Pro Val Ala Cys Ala Ala Ala Leu Ala Asn Ile Glu Leu Met Glu Arg
        355                 360                 365

gag ggc atc gtc gat cag gcg cgc gag atg gcg gac tat ttc gcc gcg  1152
Glu Gly Ile Val Asp Gln Ala Arg Glu Met Ala Asp Tyr Phe Ala Ala
    370                 375                 380

gcg ctg gct tcg ctg cgc gat ctg ccg ggc gtg gcg gaa acc cgg tcg  1200
Ala Leu Ala Ser Leu Arg Asp Leu Pro Gly Val Ala Glu Thr Arg Ser
385                 390                 395                 400

gtg ggc ctc gtg ggt tgc gtg caa tgc ctg ctc gac ccg acc cgg gcg  1248
Val Gly Leu Val Gly Cys Val Gln Cys Leu Leu Asp Pro Thr Arg Ala
                405                 410                 415

gac ggc acg gcc gag gac aag gcc ttc acc ctg aag atc gac gag cgc  1296
Asp Gly Thr Ala Glu Asp Lys Ala Phe Thr Leu Lys Ile Asp Glu Arg
            420                 425                 430

tgc ttc gag ctc ggg ctg atc gtg cgc ccg ctg ggc gat ctc tgc gtg  1344
Cys Phe Glu Leu Gly Leu Ile Val Arg Pro Leu Gly Asp Leu Cys Val
        435                 440                 445

atc tcg ccg ccg ctc atc atc tcg cgc gcg cag atc gac gag atg gtc  1392
Ile Ser Pro Pro Leu Ile Ile Ser Arg Ala Gln Ile Asp Glu Met Val
    450                 455                 460

gcg atc atg cgg cag gcc atc acc gaa gtg agc gcc gcc cac ggt ctg  1440
Ala Ile Met Arg Gln Ala Ile Thr Glu Val Ser Ala Ala His Gly Leu
465                 470                 475                 480

acc gcg aaa gaa ccg gcc gcc gtc tga                             1467
Thr Ala Lys Glu Pro Ala Ala Val
                485
```

<210> 19
<211> 488
<212> PRT
<213> 球狀紅桿菌

<400> 19

```
Met Pro Gly Cys Gly Gly Leu Pro Gly Asn Glu Pro Lys Cys Gly Arg
1               5                   10                  15
```

Glu Gly Arg Ser Ala Met Thr Arg Asn Asp Ala Thr Asn Ala Ala Gly
20 25 30

Ala Val Gly Ala Ala Met Arg Asp His Ile Leu Leu Pro Ala Gln Glu
35 40 45

Met Ala Lys Leu Gly Lys Ser Ala Gln Pro Val Leu Thr His Ala Glu
50 55 60

Gly Ile Tyr Val His Thr Glu Asp Gly Arg Arg Leu Ile Asp Gly Pro
65 70 75 80

Ala Gly Met Trp Cys Ala Gln Val Gly Tyr Gly Arg Arg Glu Ile Val
85 90 95

Asp Ala Met Ala His Gln Ala Met Val Leu Pro Tyr Ala Ser Pro Trp
100 105 110

Tyr Met Ala Thr Ser Pro Ala Ala Arg Leu Ala Glu Lys Ile Ala Thr
115 120 125

Leu Thr Pro Gly Asp Leu Asn Arg Ile Phe Phe Thr Thr Gly Gly Ser
130 135 140

Thr Ala Val Asp Ser Ala Leu Arg Phe Ser Glu Phe Tyr Asn Asn Val
145 150 155 160

Leu Gly Arg Pro Gln Lys Lys Arg Ile Ile Val Arg Tyr Asp Gly Tyr
165 170 175

His Gly Ser Thr Ala Leu Thr Ala Ala Cys Thr Gly Arg Thr Gly Asn
180 185 190

Trp Pro Asn Phe Asp Ile Ala Gln Asp Arg Ile Ser Phe Leu Ser Ser
195 200 205

Pro Asn Pro Arg His Ala Gly Asn Arg Ser Gln Glu Ala Phe Leu Asp
210 215 220

Asp Leu Val Gln Glu Phe Glu Asp Arg Ile Glu Ser Leu Gly Pro Asp
225 230 235 240

Thr Ile Ala Ala Phe Leu Ala Glu Pro Ile Leu Ala Ser Gly Gly Val
245 250 255

Ile Ile Pro Pro Ala Gly Tyr His Ala Arg Phe Lys Ala Ile Cys Glu
260 265 270

Lys His Asp Ile Leu Tyr Ile Ser Asp Glu Val Val Thr Gly Phe Gly
275 280 285

Arg Cys Gly Glu Trp Phe Ala Ser Glu Lys Val Phe Gly Val Val Pro
290 295 300

Asp Ile Ile Thr Phe Ala Lys Gly Val Thr Ser Gly Tyr Val Pro Leu
305 310 315 320

Gly Gly Leu Ala Ile Ser Glu Ala Val Leu Ala Arg Ile Ser Gly Glu
325 330 335

Asn Ala Lys Gly Ser Trp Phe Thr Asn Gly Tyr Thr Tyr Ser Asn Gln
340 345 350

Pro Val Ala Cys Ala Ala Ala Leu Ala Asn Ile Glu Leu Met Glu Arg
355 360 365

Glu Gly Ile Val Asp Gln Ala Arg Glu Met Ala Asp Tyr Phe Ala Ala
370 375 380

Ala Leu Ala Ser Leu Arg Asp Leu Pro Gly Val Ala Glu Thr Arg Ser
385 390 395 400

Val Gly Leu Val Gly Cys Val Gln Cys Leu Leu Asp Pro Thr Arg Ala
405 410 415

Asp Gly Thr Ala Glu Asp Lys Ala Phe Thr Leu Lys Ile Asp Glu Arg
420 425 430

Cys Phe Glu Leu Gly Leu Ile Val Arg Pro Leu Gly Asp Leu Cys Val
435 440 445

Ile Ser Pro Pro Leu Ile Ile Ser Arg Ala Gln Ile Asp Glu Met Val
450 455 460

Ala Ile Met Arg Gln Ala Ile Thr Glu Val Ser Ala Ala His Gly Leu
465 470 475 480

Thr Ala Lys Glu Pro Ala Ala Val
485

<210> 20
<211> 837
<212> DNA
<213> 嗜肺性退伍軍人症菌

<220>
<221> CDS
<222> (1)..(837)

<400> 20

```
atg agt atc gca ttt gtt aac ggc aag tat tgt tgt caa tct gaa gca      48
Met Ser Ile Ala Phe Val Asn Gly Lys Tyr Cys Cys Gln Ser Glu Ala
1               5                  10                  15

aaa att tca ata ttt gat cga ggg ttt ctt ttt ggt gac tcg gtt tat      96
Lys Ile Ser Ile Phe Asp Arg Gly Phe Leu Phe Gly Asp Ser Val Tyr
            20                  25                  30

gaa gtg ctg cct gtt tac cat ggg cag cct tac ttt gta gac caa cat     144
Glu Val Leu Pro Val Tyr His Gly Gln Pro Tyr Phe Val Asp Gln His
        35                  40                  45

ctt gac cga tta ttc tca aat atg aaa aaa att aag atg att ata cca     192
Leu Asp Arg Leu Phe Ser Asn Met Lys Lys Ile Lys Met Ile Ile Pro
    50                  55                  60

aat tat gat tgg cat ggt tta att cat aga cta ata tca gaa aat aat     240
Asn Tyr Asp Trp His Gly Leu Ile His Arg Leu Ile Ser Glu Asn Asn
65                  70                  75                  80

ggc ggt aat tta caa gta tat atc caa gtc aca cga ggg aat caa ggg     288
Gly Gly Asn Leu Gln Val Tyr Ile Gln Val Thr Arg Gly Asn Gln Gly
                85                  90                  95

gtg cgc aag cat gat atc cct act tcc atc aca cct tct gtt atc gca     336
Val Arg Lys His Asp Ile Pro Thr Ser Ile Thr Pro Ser Val Ile Ala
            100                 105                 110

ttc act atg cat aat cca ttt ccc acc ctc gaa gat aag gaa cag gga     384
Phe Thr Met His Asn Pro Phe Pro Thr Leu Glu Asp Lys Glu Gln Gly
        115                 120                 125

atg tca gca aaa ctg gtt gaa gat ttt cgg tgg atg aga tgt gat ata     432
Met Ser Ala Lys Leu Val Glu Asp Phe Arg Trp Met Arg Cys Asp Ile
    130                 135                 140

aaa act act tct tta att gcc aat ata tta ctg aat gat gag gct gta     480
Lys Thr Thr Ser Leu Ile Ala Asn Ile Leu Leu Asn Asp Glu Ala Val
145                 150                 155                 160

tct gca gga ttc cac act gca att ctt gcc cgg aac ggt cta att aca     528
Ser Ala Gly Phe His Thr Ala Ile Leu Ala Arg Asn Gly Leu Ile Thr
                165                 170                 175

gag gga agt agt acc aac gta ttt att gtc gca cag gat ggt gtt att     576
Glu Gly Ser Ser Thr Asn Val Phe Ile Val Ala Gln Asp Gly Val Ile
```

180 185 190

aag aca cca ccc atg aat aat ttc tgt tta cca gga att act cgg caa 624
Lys Thr Pro Pro Met Asn Asn Phe Cys Leu Pro Gly Ile Thr Arg Gln
195 200 205

gtt gtt att gaa ata att aaa aaa tta gat tta aag ttc aga gaa ata 672
Val Val Ile Glu Ile Ile Lys Lys Leu Asp Leu Lys Phe Arg Glu Ile
210 215 220

gaa att agc att tca gag ctt ttt tct gct cag gaa gtt tgg ata aca 720
Glu Ile Ser Ile Ser Glu Leu Phe Ser Ala Gln Glu Val Trp Ile Thr
225 230 235 240

agt acg aca aaa gaa gta ttc cct att aca aag att aat gac tct ttg 768
Ser Thr Thr Lys Glu Val Phe Pro Ile Thr Lys Ile Asn Asp Ser Leu
245 250 255

att aat ggc gga aaa gtt ggc gaa tat tgg cgg ata att aat gat tcc 816
Ile Asn Gly Gly Lys Val Gly Glu Tyr Trp Arg Ile Ile Asn Asp Ser
260 265 270

tac caa caa cta gta aac taa 837
Tyr Gln Gln Leu Val Asn
275

<210> 21
<211> 278
<212> PRT
<213> 嗜肺性退伍軍人症菌

<400> 21

Met Ser Ile Ala Phe Val Asn Gly Lys Tyr Cys Cys Gln Ser Glu Ala
1 5 10 15

Lys Ile Ser Ile Phe Asp Arg Gly Phe Leu Phe Gly Asp Ser Val Tyr
20 25 30

Glu Val Leu Pro Val Tyr His Gly Gln Pro Tyr Phe Val Asp Gln His
35 40 45

Leu Asp Arg Leu Phe Ser Asn Met Lys Lys Ile Lys Met Ile Ile Pro
50 55 60

Asn Tyr Asp Trp His Gly Leu Ile His Arg Leu Ile Ser Glu Asn Asn
65 70 75 80

Gly Gly Asn Leu Gln Val Tyr Ile Gln Val Thr Arg Gly Asn Gln Gly
85 90 95

Val Arg Lys His Asp Ile Pro Thr Ser Ile Thr Pro Ser Val Ile Ala

100 105 110

Phe Thr Met His Asn Pro Phe Pro Thr Leu Glu Asp Lys Glu Gln Gly
115 120 125

Met Ser Ala Lys Leu Val Glu Asp Phe Arg Trp Met Arg Cys Asp Ile
130 135 140

Lys Thr Thr Ser Leu Ile Ala Asn Ile Leu Leu Asn Asp Glu Ala Val
145 150 155 160

Ser Ala Gly Phe His Thr Ala Ile Leu Ala Arg Asn Gly Leu Ile Thr
165 170 175

Glu Gly Ser Ser Thr Asn Val Phe Ile Val Ala Gln Asp Gly Val Ile
180 185 190

Lys Thr Pro Pro Met Asn Asn Phe Cys Leu Pro Gly Ile Thr Arg Gln
195 200 205

Val Val Ile Glu Ile Ile Lys Lys Leu Asp Leu Lys Phe Arg Glu Ile
210 215 220

Glu Ile Ser Ile Ser Glu Leu Phe Ser Ala Gln Glu Val Trp Ile Thr
225 230 235 240

Ser Thr Thr Lys Glu Val Phe Pro Ile Thr Lys Ile Asn Asp Ser Leu
245 250 255

Ile Asn Gly Gly Lys Val Gly Glu Tyr Trp Arg Ile Ile Asn Asp Ser
260 265 270

Tyr Gln Gln Leu Val Asn
275

<210> 22
<211> 861
<212> DNA
<213> 歐洲亞硝酸菌

<220>
<221> CDS
<222> (1)..(861)

<400> 22
atg att tac ctc aat ggc aaa ttt ctg ccg atg gaa cag gct acc gtt 48

```
Met Ile Tyr Leu Asn Gly Lys Phe Leu Pro Met Glu Gln Ala Thr Val
1               5                  10                  15

cca gtg ctg gat aga ggc ttc atc ttc ggt gat ggt gtc tat gaa gtc      96
Pro Val Leu Asp Arg Gly Phe Ile Phe Gly Asp Gly Val Tyr Glu Val
            20                  25                  30

ata ccg gtt tat tca cgt aaa ccg ttc cgg ctg ggc gaa cat ctt tcc     144
Ile Pro Val Tyr Ser Arg Lys Pro Phe Arg Leu Gly Glu His Leu Ser
        35                  40                  45

cgg ctg cag cac agt ctg gat ggc ata cgt ctc cag aat ccg cac act     192
Arg Leu Gln His Ser Leu Asp Gly Ile Arg Leu Gln Asn Pro His Thr
    50                  55                  60

gaa gaa caa tgg gct ggt ctg atc gaa cgc atc atc gag ctg aat gaa     240
Glu Glu Gln Trp Ala Gly Leu Ile Glu Arg Ile Ile Glu Leu Asn Glu
65                  70                  75                  80

ggt gat gat cag tac ctt tac ctg cac att aca cgc ggg gtg gca aaa     288
Gly Asp Asp Gln Tyr Leu Tyr Leu His Ile Thr Arg Gly Val Ala Lys
                85                  90                  95

cgt gac cat gcc ttt cct cgc gaa gta acg ccc act gtc ttc atc atg     336
Arg Asp His Ala Phe Pro Arg Glu Val Thr Pro Thr Val Phe Ile Met
            100                 105                 110

agc aac ccg ctt ccg gct cca cct gca aaa ttg ctc gtt tcc gga gtt     384
Ser Asn Pro Leu Pro Ala Pro Pro Ala Lys Leu Leu Val Ser Gly Val
        115                 120                 125

tca gcg att acc gcc agg gat aat cgc tgg ggg cgc tgt gat atc aaa     432
Ser Ala Ile Thr Ala Arg Asp Asn Arg Trp Gly Arg Cys Asp Ile Lys
    130                 135                 140

gcc att tca ctg ttg cca aat atc tta ttg cgc cag ctt gcc gtg gac     480
Ala Ile Ser Leu Leu Pro Asn Ile Leu Leu Arg Gln Leu Ala Val Asp
145                 150                 155                 160

gca caa gcc atg gaa acg atc ctg tta cgc gat ggt ctg ttg acc gaa     528
Ala Gln Ala Met Glu Thr Ile Leu Leu Arg Asp Gly Leu Leu Thr Glu
                165                 170                 175

ggg gcc gcc agc aat att ttc atc gta aaa gac gac ctg ctg ctg acc     576
Gly Ala Ala Ser Asn Ile Phe Ile Val Lys Asp Asp Leu Leu Leu Thr
            180                 185                 190

ccc ccc aaa gat cac cgt ata ttg cct ggc att act tat gat gta gta     624
Pro Pro Lys Asp His Arg Ile Leu Pro Gly Ile Thr Tyr Asp Val Val
        195                 200                 205

ctg gaa ctg gct gaa aca cat ggt gtt cca cat gcg aca aga gaa ata     672
Leu Glu Leu Ala Glu Thr His Gly Val Pro His Ala Thr Arg Glu Ile
    210                 215                 220

tca gag ctt gag tta cgt act gca cgg gaa atc atg ctg act tct tcc     720
Ser Glu Leu Glu Leu Arg Thr Ala Arg Glu Ile Met Leu Thr Ser Ser
225                 230                 235                 240
```

acc aaa gaa att ctc ccg atc aca cag ctg gat gga caa ccg atc ggt 768
Thr Lys Glu Ile Leu Pro Ile Thr Gln Leu Asp Gly Gln Pro Ile Gly
245 250 255

aat ggc acc cca ggg cca gta ttt cag caa ctg gat cgg ctc tat cag 816
Asn Gly Thr Pro Gly Pro Val Phe Gln Gln Leu Asp Arg Leu Tyr Gln
260 265 270

gca tat aag ctg gaa gtc atg cgc ggg cat gct cca cgc cag taa 861
Ala Tyr Lys Leu Glu Val Met Arg Gly His Ala Pro Arg Gln
275 280 285

<210> 23
<211> 286
<212> PRT
<213> 歐洲亞硝酸菌

<400> 23

Met Ile Tyr Leu Asn Gly Lys Phe Leu Pro Met Glu Gln Ala Thr Val
1 5 10 15

Pro Val Leu Asp Arg Gly Phe Ile Phe Gly Asp Gly Val Tyr Glu Val
20 25 30

Ile Pro Val Tyr Ser Arg Lys Pro Phe Arg Leu Gly Glu His Leu Ser
35 40 45

Arg Leu Gln His Ser Leu Asp Gly Ile Arg Leu Gln Asn Pro His Thr
50 55 60

Glu Glu Gln Trp Ala Gly Leu Ile Glu Arg Ile Ile Glu Leu Asn Glu
65 70 75 80

Gly Asp Asp Gln Tyr Leu Tyr Leu His Ile Thr Arg Gly Val Ala Lys
85 90 95

Arg Asp His Ala Phe Pro Arg Glu Val Thr Pro Thr Val Phe Ile Met
100 105 110

Ser Asn Pro Leu Pro Ala Pro Pro Ala Lys Leu Leu Val Ser Gly Val
115 120 125

Ser Ala Ile Thr Ala Arg Asp Asn Arg Trp Gly Arg Cys Asp Ile Lys
130 135 140

Ala Ile Ser Leu Leu Pro Asn Ile Leu Leu Arg Gln Leu Ala Val Asp
145 150 155 160

Ala Gln Ala Met Glu Thr Ile Leu Leu Arg Asp Gly Leu Leu Thr Glu
165 170 175

Gly Ala Ala Ser Asn Ile Phe Ile Val Lys Asp Asp Leu Leu Leu Thr
180 185 190

Pro Pro Lys Asp His Arg Ile Leu Pro Gly Ile Thr Tyr Asp Val Val
195 200 205

Leu Glu Leu Ala Glu Thr His Gly Val Pro His Ala Thr Arg Glu Ile
210 215 220

Ser Glu Leu Glu Leu Arg Thr Ala Arg Glu Ile Met Leu Thr Ser Ser
225 230 235 240

Thr Lys Glu Ile Leu Pro Ile Thr Gln Leu Asp Gly Gln Pro Ile Gly
245 250 255

Asn Gly Thr Pro Gly Pro Val Phe Gln Gln Leu Asp Arg Leu Tyr Gln
260 265 270

Ala Tyr Lys Leu Glu Val Met Arg Gly His Ala Pro Arg Gln
275 280 285

<210> 24
<211> 1293
<212> DNA
<213> 淋病奈瑟氏菌

<220>
<221> CDS
<222> (1)..(1293)

<400> 24

atg agg ata aat atg aac cgt aac gaa att tta ttc gac cgc gcc aag 48
Met Arg Ile Asn Met Asn Arg Asn Glu Ile Leu Phe Asp Arg Ala Lys
1 5 10 15

gcc atc atc ccc ggc ggc gtg aat tcg ccc gtg cgc gca ttc ggc agc 96
Ala Ile Ile Pro Gly Gly Val Asn Ser Pro Val Arg Ala Phe Gly Ser
20 25 30

gtc ggc ggc gtg ccg cgc ttc atc aaa aaa gcc gaa ggc gcg tat gtt 144
Val Gly Gly Val Pro Arg Phe Ile Lys Lys Ala Glu Gly Ala Tyr Val
35 40 45

tgg gac gaa aac ggc acg cgc tac acc gat tat gtc ggc tct tgg ggg 192
Trp Asp Glu Asn Gly Thr Arg Tyr Thr Asp Tyr Val Gly Ser Trp Gly
50 55 60

```
cct gcg att gtc gga cac gcg cat ccc gaa gtc gtc gaa gcc gtg cgc      240
Pro Ala Ile Val Gly His Ala His Pro Glu Val Val Glu Ala Val Arg
65                  70                  75                  80

gaa gct gcg ttg ggc ggt ttg tcg ttc ggc gcg ccc acc gaa ggc gaa      288
Glu Ala Ala Leu Gly Gly Leu Ser Phe Gly Ala Pro Thr Glu Gly Glu
                85                  90                  95

atc gcc att gcc gaa caa att gcc gaa att atg ccg tct gtc gaa cgg      336
Ile Ala Ile Ala Glu Gln Ile Ala Glu Ile Met Pro Ser Val Glu Arg
            100                 105                 110

ctg cgc ctc gtc agc tcc ggc acg gaa gcg acg atg act gcc atc cgt      384
Leu Arg Leu Val Ser Ser Gly Thr Glu Ala Thr Met Thr Ala Ile Arg
        115                 120                 125

ctg gca cgc ggt ttt acc ggc cgc gac aaa atc atc aaa ttt gaa ggc      432
Leu Ala Arg Gly Phe Thr Gly Arg Asp Lys Ile Ile Lys Phe Glu Gly
    130                 135                 140

tgc tac cac ggc cat tcc gac agc ctg ttg gtg aaa gca ggc agc ggt      480
Cys Tyr His Gly His Ser Asp Ser Leu Leu Val Lys Ala Gly Ser Gly
145                 150                 155                 160

ctg ctt acc ttc ggc aat cct tct tcc gcc ggt gtg cct gcc gac ttt      528
Leu Leu Thr Phe Gly Asn Pro Ser Ser Ala Gly Val Pro Ala Asp Phe
                165                 170                 175

acc aaa cat act ttg gta ctc gaa tac aac aac atc gcc caa ctc gaa      576
Thr Lys His Thr Leu Val Leu Glu Tyr Asn Asn Ile Ala Gln Leu Glu
            180                 185                 190

gaa gcc ttt gcc caa agc ggc gac gaa atc gcc tgc gtg att gtc gaa      624
Glu Ala Phe Ala Gln Ser Gly Asp Glu Ile Ala Cys Val Ile Val Glu
        195                 200                 205

ccc ttc gtc ggc aat atg aac ctc gtc cgc ccg acc gaa gcc ttt gtc      672
Pro Phe Val Gly Asn Met Asn Leu Val Arg Pro Thr Glu Ala Phe Val
    210                 215                 220

aaa gcc ttg cgc gga ttg acc gaa aaa cac ggc gcg gtg ttg att tac      720
Lys Ala Leu Arg Gly Leu Thr Glu Lys His Gly Ala Val Leu Ile Tyr
225                 230                 235                 240

gac gaa gtg atg acc ggt ttc cgc gtc gcg ctc ggc ggc gcg cag tcg      768
Asp Glu Val Met Thr Gly Phe Arg Val Ala Leu Gly Gly Ala Gln Ser
                245                 250                 255

ctg cac ggc atc acg ccc gac ctg acc acg atg ggc aaa gtc atc ggc      816
Leu His Gly Ile Thr Pro Asp Leu Thr Thr Met Gly Lys Val Ile Gly
            260                 265                 270

ggc ggt atg ccg ctt gcc gcg ttc ggc gga cgc aaa gac atc atg gaa      864
Gly Gly Met Pro Leu Ala Ala Phe Gly Gly Arg Lys Asp Ile Met Glu
        275                 280                 285

tgt att tcc ccg ttg ggc ggc gtg tat cag gca ggt aca tta tca ggc      912
Cys Ile Ser Pro Leu Gly Gly Val Tyr Gln Ala Gly Thr Leu Ser Gly
```

```
    290                 295                 300

aac ccg att gcc gtc gcc gcc ggc ttg aaa acg ctg gaa atc atc cag      960
Asn Pro Ile Ala Val Ala Ala Gly Leu Lys Thr Leu Glu Ile Ile Gln
305                 310                 315                 320

cgc gaa ggc ttc tat gaa aac ctg acc gcc ttg aca caa cgc ctt gcc     1008
Arg Glu Gly Phe Tyr Glu Asn Leu Thr Ala Leu Thr Gln Arg Leu Ala
                325                 330                 335

aac ggt att gcc gcc gcc aaa gcg cac ggt atc gag ttt gcc gcc gac     1056
Asn Gly Ile Ala Ala Ala Lys Ala His Gly Ile Glu Phe Ala Ala Asp
            340                 345                 350

agc gtg ggc ggt atg ttc ggt ctg tat ttc gcc gca cac gtg ccg cga     1104
Ser Val Gly Gly Met Phe Gly Leu Tyr Phe Ala Ala His Val Pro Arg
        355                 360                 365

aac tat gcc gat atg gcg cgc tcc aat atc gac gct ttc aaa cgc ttc     1152
Asn Tyr Ala Asp Met Ala Arg Ser Asn Ile Asp Ala Phe Lys Arg Phe
    370                 375                 380

ttc cac ggc atg ctc gac cgc ggc att gcc ttc ggc ccg tcc gct tat     1200
Phe His Gly Met Leu Asp Arg Gly Ile Ala Phe Gly Pro Ser Ala Tyr
385                 390                 395                 400

gaa gcg ggt ttc gtt tcc gcc gcg cat acg ccc gag ctg att gac gaa     1248
Glu Ala Gly Phe Val Ser Ala Ala His Thr Pro Glu Leu Ile Asp Glu
                405                 410                 415

acg gtt gcg gtt gcg gtt gaa gtg ttc aag gcg atg gct gca tga          1293
Thr Val Ala Val Ala Val Glu Val Phe Lys Ala Met Ala Ala
            420                 425                 430
```

<210> 25
<211> 430
<212> PRT
<213> 淋病奈瑟氏菌

<400> 25

```
Met Arg Ile Asn Met Asn Arg Asn Glu Ile Leu Phe Asp Arg Ala Lys
1               5                   10                  15

Ala Ile Ile Pro Gly Gly Val Asn Ser Pro Val Arg Ala Phe Gly Ser
            20                  25                  30

Val Gly Gly Val Pro Arg Phe Ile Lys Lys Ala Glu Gly Ala Tyr Val
        35                  40                  45

Trp Asp Glu Asn Gly Thr Arg Tyr Thr Asp Tyr Val Gly Ser Trp Gly
    50                  55                  60

Pro Ala Ile Val Gly His Ala His Pro Glu Val Val Glu Ala Val Arg
```

65 70 75 80

Glu Ala Ala Leu Gly Gly Leu Ser Phe Gly Ala Pro Thr Glu Gly Glu
85 90 95

Ile Ala Ile Ala Glu Gln Ile Ala Glu Ile Met Pro Ser Val Glu Arg
100 105 110

Leu Arg Leu Val Ser Ser Gly Thr Glu Ala Thr Met Thr Ala Ile Arg
115 120 125

Leu Ala Arg Gly Phe Thr Gly Arg Asp Lys Ile Ile Lys Phe Glu Gly
130 135 140

Cys Tyr His Gly His Ser Asp Ser Leu Leu Val Lys Ala Gly Ser Gly
145 150 155 160

Leu Leu Thr Phe Gly Asn Pro Ser Ser Ala Gly Val Pro Ala Asp Phe
165 170 175

Thr Lys His Thr Leu Val Leu Glu Tyr Asn Asn Ile Ala Gln Leu Glu
180 185 190

Glu Ala Phe Ala Gln Ser Gly Asp Glu Ile Ala Cys Val Ile Val Glu
195 200 205

Pro Phe Val Gly Asn Met Asn Leu Val Arg Pro Thr Glu Ala Phe Val
210 215 220

Lys Ala Leu Arg Gly Leu Thr Glu Lys His Gly Ala Val Leu Ile Tyr
225 230 235 240

Asp Glu Val Met Thr Gly Phe Arg Val Ala Leu Gly Gly Ala Gln Ser
245 250 255

Leu His Gly Ile Thr Pro Asp Leu Thr Thr Met Gly Lys Val Ile Gly
260 265 270

Gly Gly Met Pro Leu Ala Ala Phe Gly Gly Arg Lys Asp Ile Met Glu
275 280 285

Cys Ile Ser Pro Leu Gly Gly Val Tyr Gln Ala Gly Thr Leu Ser Gly
290 295 300

Asn Pro Ile Ala Val Ala Ala Gly Leu Lys Thr Leu Glu Ile Ile Gln
305 310 315 320

Arg Glu Gly Phe Tyr Glu Asn Leu Thr Ala Leu Thr Gln Arg Leu Ala
325 330 335

Asn Gly Ile Ala Ala Ala Lys Ala His Gly Ile Glu Phe Ala Ala Asp
340 345 350

Ser Val Gly Gly Met Phe Gly Leu Tyr Phe Ala Ala His Val Pro Arg
355 360 365

Asn Tyr Ala Asp Met Ala Arg Ser Asn Ile Asp Ala Phe Lys Arg Phe
370 375 380

Phe His Gly Met Leu Asp Arg Gly Ile Ala Phe Gly Pro Ser Ala Tyr
385 390 395 400

Glu Ala Gly Phe Val Ser Ala Ala His Thr Pro Glu Leu Ile Asp Glu
405 410 415

Thr Val Ala Val Ala Val Glu Val Phe Lys Ala Met Ala Ala
420 425 430

<210> 26
<211> 924
<212> DNA
<213> 綠膿桿菌(gi 9951299)

<220>
<221> CDS
<222> (1)..(924)

<400> 26
atg tcg atg gcc gat cgt gat ggc gtg atc tgg tat gac ggt gaa ctg 48
Met Ser Met Ala Asp Arg Asp Gly Val Ile Trp Tyr Asp Gly Glu Leu
1 5 10 15

gtg cag tgg cgc gac gcg acc acg cac gtg ctg acc cat acc ctg cac 96
Val Gln Trp Arg Asp Ala Thr Thr His Val Leu Thr His Thr Leu His
20 25 30

tat gga atg ggc gtg ttc gag ggc gtg cgc gcc tac gac acc ccg cag 144
Tyr Gly Met Gly Val Phe Glu Gly Val Arg Ala Tyr Asp Thr Pro Gln
35 40 45

ggc acg gcg atc ttc cgc ctg cag gcg cat acc gac cgg ctg ttc gac 192
Gly Thr Ala Ile Phe Arg Leu Gln Ala His Thr Asp Arg Leu Phe Asp
50 55 60

```
tcc gcg cac atc atg aac atg cag atc ccg tac agc cgc gac gag atc      240
Ser Ala His Ile Met Asn Met Gln Ile Pro Tyr Ser Arg Asp Glu Ile
65                  70                  75                  80

aac gag gcg acc cgc gcc gcc gtg cgc gag aac aac ctg gaa agc gcc      288
Asn Glu Ala Thr Arg Ala Ala Val Arg Glu Asn Asn Leu Glu Ser Ala
                85                  90                  95

tat atc cgc ccg atg gtg ttc tac gga agc gaa ggc atg ggc ctg cgc      336
Tyr Ile Arg Pro Met Val Phe Tyr Gly Ser Glu Gly Met Gly Leu Arg
            100                 105                 110

gcc agc ggc ctg aag gtc cat gtg atc atc gcc gcc tgg agc tgg ggc      384
Ala Ser Gly Leu Lys Val His Val Ile Ile Ala Ala Trp Ser Trp Gly
        115                 120                 125

gcc tac atg ggc gag gaa gcc ctg cag caa ggc atc aag gtg cgc acc      432
Ala Tyr Met Gly Glu Glu Ala Leu Gln Gln Gly Ile Lys Val Arg Thr
    130                 135                 140

agt tcc ttc acc cgc cac cac gtc aac atc tcg atg acc cgc gcc aag      480
Ser Ser Phe Thr Arg His His Val Asn Ile Ser Met Thr Arg Ala Lys
145                 150                 155                 160

tcc aac ggc gcc tac atc aac tcg atg ctg gcc ctc cag gaa gcg atc      528
Ser Asn Gly Ala Tyr Ile Asn Ser Met Leu Ala Leu Gln Glu Ala Ile
                165                 170                 175

tcc ggc ggc gcc gac gag gcc atg atg ctc gat ccg gaa ggc tac gtg      576
Ser Gly Gly Ala Asp Glu Ala Met Met Leu Asp Pro Glu Gly Tyr Val
            180                 185                 190

gcc gaa ggc tcc ggc gag aac atc ttc atc atc aag gat ggc gtg atc      624
Ala Glu Gly Ser Gly Glu Asn Ile Phe Ile Ile Lys Asp Gly Val Ile
        195                 200                 205

tac acc ccg gaa gtc acc gcc tgc ctg aac ggc atc act cgt aac act      672
Tyr Thr Pro Glu Val Thr Ala Cys Leu Asn Gly Ile Thr Arg Asn Thr
    210                 215                 220

atc ctg acc ctg gcc gcc gaa cac ggt ttt aaa ctg gtc gag aag cgc      720
Ile Leu Thr Leu Ala Ala Glu His Gly Phe Lys Leu Val Glu Lys Arg
225                 230                 235                 240

atc acc cgc gac gag gtg tac atc gcc gac gag gcc ttc ttc act ggc      768
Ile Thr Arg Asp Glu Val Tyr Ile Ala Asp Glu Ala Phe Phe Thr Gly
                245                 250                 255

act gcc gcg gaa gtc acg ccg atc cgc gaa gtg gac ggt cgc aag atc      816
Thr Ala Ala Glu Val Thr Pro Ile Arg Glu Val Asp Gly Arg Lys Ile
            260                 265                 270

ggc gcc ggc cgc cgt ggc ccg gtc acc gaa aag ctg cag aaa gcc tat      864
Gly Ala Gly Arg Arg Gly Pro Val Thr Glu Lys Leu Gln Lys Ala Tyr
        275                 280                 285

ttc gac ctg gtc agc ggc aag acc gag gcc cac gcc gag tgg cgt acc      912
Phe Asp Leu Val Ser Gly Lys Thr Glu Ala His Ala Glu Trp Arg Thr
    290                 295                 300
```

ctg gtc aag taa 924
Leu Val Lys
305

<210> 27
<211> 307
<212> PRT
<213> 綠膿桿菌(gi 9951299)

<400> 27

Met Ser Met Ala Asp Arg Asp Gly Val Ile Trp Tyr Asp Gly Glu Leu
1 5 10 15

Val Gln Trp Arg Asp Ala Thr Thr His Val Leu Thr His Thr Leu His
20 25 30

Tyr Gly Met Gly Val Phe Glu Gly Val Arg Ala Tyr Asp Thr Pro Gln
35 40 45

Gly Thr Ala Ile Phe Arg Leu Gln Ala His Thr Asp Arg Leu Phe Asp
50 55 60

Ser Ala His Ile Met Asn Met Gln Ile Pro Tyr Ser Arg Asp Glu Ile
65 70 75 80

Asn Glu Ala Thr Arg Ala Ala Val Arg Glu Asn Asn Leu Glu Ser Ala
85 90 95

Tyr Ile Arg Pro Met Val Phe Tyr Gly Ser Glu Gly Met Gly Leu Arg
100 105 110

Ala Ser Gly Leu Lys Val His Val Ile Ile Ala Ala Trp Ser Trp Gly
115 120 125

Ala Tyr Met Gly Glu Glu Ala Leu Gln Gln Gly Ile Lys Val Arg Thr
130 135 140

Ser Ser Phe Thr Arg His His Val Asn Ile Ser Met Thr Arg Ala Lys
145 150 155 160

Ser Asn Gly Ala Tyr Ile Asn Ser Met Leu Ala Leu Gln Glu Ala Ile
165 170 175

Ser Gly Gly Ala Asp Glu Ala Met Met Leu Asp Pro Glu Gly Tyr Val
180 185 190

Ala Glu Gly Ser Gly Glu Asn Ile Phe Ile Ile Lys Asp Gly Val Ile
195 200 205

Tyr Thr Pro Glu Val Thr Ala Cys Leu Asn Gly Ile Thr Arg Asn Thr
210 215 220

Ile Leu Thr Leu Ala Ala Glu His Gly Phe Lys Leu Val Glu Lys Arg
225 230 235 240

Ile Thr Arg Asp Glu Val Tyr Ile Ala Asp Glu Ala Phe Phe Thr Gly
245 250 255

Thr Ala Ala Glu Val Thr Pro Ile Arg Glu Val Asp Gly Arg Lys Ile
260 265 270

Gly Ala Gly Arg Arg Gly Pro Val Thr Glu Lys Leu Gln Lys Ala Tyr
275 280 285

Phe Asp Leu Val Ser Gly Lys Thr Glu Ala His Ala Glu Trp Arg Thr
290 295 300

Leu Val Lys
305

<210> 28
<211> 1407
<212> DNA
<213> 沼澤紅假單胞菌

<220>
<221> CDS
<222> (1)..(1407)

<400> 28
atg aag ctg ata ccg tgc cgc gcc ttt cac ccc ccg gcc gcg cag tgc 48
Met Lys Leu Ile Pro Cys Arg Ala Phe His Pro Pro Ala Ala Gln Cys
1 5 10 15

atg agg agc gcc atg tta gac aag atc aag ccc acg tcc gcc gtc aac 96
Met Arg Ser Ala Met Leu Asp Lys Ile Lys Pro Thr Ser Ala Val Asn
20 25 30

gcg ccg aac gat ctc aac gcg ttc tgg atg ccg ttc acc gcg aac cgg 144
Ala Pro Asn Asp Leu Asn Ala Phe Trp Met Pro Phe Thr Ala Asn Arg
35 40 45

gcc ttc aag cgc gcg ccg aag atg gtc gtg ggt gcc gaa ggc atg cac 192
Ala Phe Lys Arg Ala Pro Lys Met Val Val Gly Ala Glu Gly Met His

```
    50                  55                  60

tac atc acc gcc gat ggt cgc aag atc atc gac gcc gcc tcg ggc atg      240
Tyr Ile Thr Ala Asp Gly Arg Lys Ile Ile Asp Ala Ala Ser Gly Met
65                  70                  75                  80

tgg tgc acc aat gcg ggc cat ggc cgc aag gaa atc gcc gag gcg atc      288
Trp Cys Thr Asn Ala Gly His Gly Arg Lys Glu Ile Ala Glu Ala Ile
                85                  90                  95

aag gcg cag gcc gat gaa ctc gac ttc tcg ccg ccg ttc cag ttc ggc      336
Lys Ala Gln Ala Asp Glu Leu Asp Phe Ser Pro Pro Phe Gln Phe Gly
            100                 105                 110

cag ccg aag gcg ttc gaa ctc gcc agc cgg atc gcc gat ctg gcg ccg      384
Gln Pro Lys Ala Phe Glu Leu Ala Ser Arg Ile Ala Asp Leu Ala Pro
        115                 120                 125

gaa ggc ctc gat cac gtg ttc ttc tgc aat tcg ggc tcg gaa gcc ggc      432
Glu Gly Leu Asp His Val Phe Phe Cys Asn Ser Gly Ser Glu Ala Gly
    130                 135                 140

gac acc gcg ctg aag atc gcg gtc gcc tat cag cag atc aag ggc cag      480
Asp Thr Ala Leu Lys Ile Ala Val Ala Tyr Gln Gln Ile Lys Gly Gln
145                 150                 155                 160

ggc tca cgc acc cgc ctg atc ggc cgc gag cgc ggc tat cac ggc gtc      528
Gly Ser Arg Thr Arg Leu Ile Gly Arg Glu Arg Gly Tyr His Gly Val
                165                 170                 175

ggc ttc ggc ggc acc gcg gtc ggc ggc atc ggc aac aac cgc aag atg      576
Gly Phe Gly Gly Thr Ala Val Gly Gly Ile Gly Asn Asn Arg Lys Met
            180                 185                 190

ttc ggt ccg ctg ctc aac ggc gtc gat cat ctg cct gcg act tat gat      624
Phe Gly Pro Leu Leu Asn Gly Val Asp His Leu Pro Ala Thr Tyr Asp
        195                 200                 205

cgc gac aag cag gct ttc acc atc ggc gag ccg gaa tac ggc gcg cac      672
Arg Asp Lys Gln Ala Phe Thr Ile Gly Glu Pro Glu Tyr Gly Ala His
    210                 215                 220

ttc gcc gaa gcg ctt gaa ggc ctc gtc aat ctg cac ggc gcc aac acc      720
Phe Ala Glu Ala Leu Glu Gly Leu Val Asn Leu His Gly Ala Asn Thr
225                 230                 235                 240

atc gcg gcg gtg atc gtc gag ccg atg gcc ggc tcc acc ggc gtg ctg      768
Ile Ala Ala Val Ile Val Glu Pro Met Ala Gly Ser Thr Gly Val Leu
                245                 250                 255

ccg gcg ccg aag ggc tat ctc aag aag ctg cgc gag atc acc aag aag      816
Pro Ala Pro Lys Gly Tyr Leu Lys Lys Leu Arg Glu Ile Thr Lys Lys
            260                 265                 270

cac ggc atc ctg ctg atc ttc gac gag gtc atc acc ggc tac ggc cgt      864
His Gly Ile Leu Leu Ile Phe Asp Glu Val Ile Thr Gly Tyr Gly Arg
        275                 280                 285

ctc ggc tat gcc ttc gcg tcc gaa cgt tac ggc gtc acc ccg gac atg      912
```

```
Leu Gly Tyr Ala Phe Ala Ser Glu Arg Tyr Gly Val Thr Pro Asp Met
    290                 295                 300

atc acc ttc gcc aag ggc gtc acc aat ggt gcg gtg ccg atg ggc ggc      960
Ile Thr Phe Ala Lys Gly Val Thr Asn Gly Ala Val Pro Met Gly Gly
305                 310                 315                 320

gtg atc acc tcg gcg gag atc cac gat gcg ttc atg acc ggc ccc gag     1008
Val Ile Thr Ser Ala Glu Ile His Asp Ala Phe Met Thr Gly Pro Glu
                325                 330                 335

cac gcg gtc gag ctg gcg cac ggc tac acc tat tcg gcg cat ccg ctc     1056
His Ala Val Glu Leu Ala His Gly Tyr Thr Tyr Ser Ala His Pro Leu
            340                 345                 350

gcc tgc gcg gcc ggc atc gcc acc ctc gac atc tac cgc gac gag aag     1104
Ala Cys Ala Ala Gly Ile Ala Thr Leu Asp Ile Tyr Arg Asp Glu Lys
        355                 360                 365

ctg ttc gag cgc gcc aag gcg ctg gag ccg aag ttt gcc gag gcg gtg     1152
Leu Phe Glu Arg Ala Lys Ala Leu Glu Pro Lys Phe Ala Glu Ala Val
    370                 375                 380

atg tcg ctg aag tcg gcc ccg aac gtg gtc gac atc cgc acc gtc ggc     1200
Met Ser Leu Lys Ser Ala Pro Asn Val Val Asp Ile Arg Thr Val Gly
385                 390                 395                 400

ctg acg gcg ggt atc gac ctc gct tcg atc gcc gat gcg gtc ggc aag     1248
Leu Thr Ala Gly Ile Asp Leu Ala Ser Ile Ala Asp Ala Val Gly Lys
                405                 410                 415

cgt ggc ttc gaa gcg atg aat gcc ggc ttc cac gac cac gag ctg atg     1296
Arg Gly Phe Glu Ala Met Asn Ala Gly Phe His Asp His Glu Leu Met
            420                 425                 430

ctg cgg atc gcc ggc gac acc ctg gcg ctg acc ccg ccg ctg atc ctc     1344
Leu Arg Ile Ala Gly Asp Thr Leu Ala Leu Thr Pro Pro Leu Ile Leu
        435                 440                 445

agc gag gac cac atc ggt gag atc gtc gac aag gtc ggc aag gtg atc     1392
Ser Glu Asp His Ile Gly Glu Ile Val Asp Lys Val Gly Lys Val Ile
    450                 455                 460

cgc gcg gtc gcc tga                                                1407
Arg Ala Val Ala
465
```

<210> 29
<211> 468
<212> PRT
<213> 沼澤紅假單胞菌

<400> 29

```
Met Lys Leu Ile Pro Cys Arg Ala Phe His Pro Pro Ala Ala Gln Cys
1               5                   10                  15
```

Met Arg Ser Ala Met Leu Asp Lys Ile Lys Pro Thr Ser Ala Val Asn
20 25 30

Ala Pro Asn Asp Leu Asn Ala Phe Trp Met Pro Phe Thr Ala Asn Arg
35 40 45

Ala Phe Lys Arg Ala Pro Lys Met Val Val Gly Ala Glu Gly Met His
50 55 60

Tyr Ile Thr Ala Asp Gly Arg Lys Ile Ile Asp Ala Ala Ser Gly Met
65 70 75 80

Trp Cys Thr Asn Ala Gly His Gly Arg Lys Glu Ile Ala Glu Ala Ile
85 90 95

Lys Ala Gln Ala Asp Glu Leu Asp Phe Ser Pro Pro Phe Gln Phe Gly
100 105 110

Gln Pro Lys Ala Phe Glu Leu Ala Ser Arg Ile Ala Asp Leu Ala Pro
115 120 125

Glu Gly Leu Asp His Val Phe Phe Cys Asn Ser Gly Ser Glu Ala Gly
130 135 140

Asp Thr Ala Leu Lys Ile Ala Val Ala Tyr Gln Gln Ile Lys Gly Gln
145 150 155 160

Gly Ser Arg Thr Arg Leu Ile Gly Arg Glu Arg Gly Tyr His Gly Val
165 170 175

Gly Phe Gly Gly Thr Ala Val Gly Gly Ile Gly Asn Asn Arg Lys Met
180 185 190

Phe Gly Pro Leu Leu Asn Gly Val Asp His Leu Pro Ala Thr Tyr Asp
195 200 205

Arg Asp Lys Gln Ala Phe Thr Ile Gly Glu Pro Glu Tyr Gly Ala His
210 215 220

Phe Ala Glu Ala Leu Glu Gly Leu Val Asn Leu His Gly Ala Asn Thr
225 230 235 240

Ile Ala Ala Val Ile Val Glu Pro Met Ala Gly Ser Thr Gly Val Leu
245 250 255

Pro Ala Pro Lys Gly Tyr Leu Lys Lys Leu Arg Glu Ile Thr Lys Lys
260 265 270

His Gly Ile Leu Leu Ile Phe Asp Glu Val Ile Thr Gly Tyr Gly Arg
275 280 285

Leu Gly Tyr Ala Phe Ala Ser Glu Arg Tyr Gly Val Thr Pro Asp Met
290 295 300

Ile Thr Phe Ala Lys Gly Val Thr Asn Gly Ala Val Pro Met Gly Gly
305 310 315 320

Val Ile Thr Ser Ala Glu Ile His Asp Ala Phe Met Thr Gly Pro Glu
325 330 335

His Ala Val Glu Leu Ala His Gly Tyr Thr Tyr Ser Ala His Pro Leu
340 345 350

Ala Cys Ala Ala Gly Ile Ala Thr Leu Asp Ile Tyr Arg Asp Glu Lys
355 360 365

Leu Phe Glu Arg Ala Lys Ala Leu Glu Pro Lys Phe Ala Glu Ala Val
370 375 380

Met Ser Leu Lys Ser Ala Pro Asn Val Val Asp Ile Arg Thr Val Gly
385 390 395 400

Leu Thr Ala Gly Ile Asp Leu Ala Ser Ile Ala Asp Ala Val Gly Lys
405 410 415

Arg Gly Phe Glu Ala Met Asn Ala Gly Phe His Asp His Glu Leu Met
420 425 430

Leu Arg Ile Ala Gly Asp Thr Leu Ala Leu Thr Pro Pro Leu Ile Leu
435 440 445

Ser Glu Asp His Ile Gly Glu Ile Val Asp Lys Val Gly Lys Val Ile
450 455 460

Arg Ala Val Ala
465

<210> 30
<211> 1263

<212> DNA
<213> 大腸桿菌

<220>
<221> CDS
<222> (1)..(1263)

<400> 30

```
atg cca cat tca ctg ttc agc acc gat acc gat ctc acc gcc gaa aat      48
Met Pro His Ser Leu Phe Ser Thr Asp Thr Asp Leu Thr Ala Glu Asn
1               5                  10                  15

ctg ctg cgt ttg ccc gct gaa ttt ggc tgc ccg gtg tgg gtc tac gat      96
Leu Leu Arg Leu Pro Ala Glu Phe Gly Cys Pro Val Trp Val Tyr Asp
            20                  25                  30

gcg caa att att cgt cgg cag att gca gcg ctg aaa cag ttt gat gtg     144
Ala Gln Ile Ile Arg Arg Gln Ile Ala Ala Leu Lys Gln Phe Asp Val
        35                  40                  45

gtg cgc ttt gca cag aaa gcc tgt tcc aat att cat att ttg cgc tta     192
Val Arg Phe Ala Gln Lys Ala Cys Ser Asn Ile His Ile Leu Arg Leu
    50                  55                  60

atg cgt gag cag ggc gtg aaa gtg gat tcc gtc tcg tta ggc gaa ata     240
Met Arg Glu Gln Gly Val Lys Val Asp Ser Val Ser Leu Gly Glu Ile
65                  70                  75                  80

gag cgt gcg ttg gcg gcg ggt tac aat ccg caa acg cac ccc gat gat     288
Glu Arg Ala Leu Ala Ala Gly Tyr Asn Pro Gln Thr His Pro Asp Asp
                85                  90                  95

att gtt ttt acg gca gat gtt atc gat cag gcg acg ctt gaa cgc gtc     336
Ile Val Phe Thr Ala Asp Val Ile Asp Gln Ala Thr Leu Glu Arg Val
            100                 105                 110

agt gaa ttg caa att ccg gtg aat gcg ggt tct gtt gat atg ctc gac     384
Ser Glu Leu Gln Ile Pro Val Asn Ala Gly Ser Val Asp Met Leu Asp
        115                 120                 125

caa ctg ggc cag gtt tcg cca ggg cat cgg gta tgg ctg cgc gtt aat     432
Gln Leu Gly Gln Val Ser Pro Gly His Arg Val Trp Leu Arg Val Asn
    130                 135                 140

ccg ggg ttt ggt cac gga cat agc caa aaa acc aat acc ggt ggc gaa     480
Pro Gly Phe Gly His Gly His Ser Gln Lys Thr Asn Thr Gly Gly Glu
145                 150                 155                 160

aac agc aag cac ggt atc tgg tac acc gat ctg ccc gcc gca ctg gac     528
Asn Ser Lys His Gly Ile Trp Tyr Thr Asp Leu Pro Ala Ala Leu Asp
                165                 170                 175

gtg ata caa cgt cat cat ctg cag ctg gtc ggc att cac atg cac att     576
Val Ile Gln Arg His His Leu Gln Leu Val Gly Ile His Met His Ile
            180                 185                 190

ggt tct ggc gtt gat tat gcc cat ctg gaa cag gtg tgt ggt gct atg     624
Gly Ser Gly Val Asp Tyr Ala His Leu Glu Gln Val Cys Gly Ala Met
```

```
                195                 200                 205

gtg cgt cag gtc atc gaa ttc ggt cag gat tta cag gct att tct gcg      672
Val Arg Gln Val Ile Glu Phe Gly Gln Asp Leu Gln Ala Ile Ser Ala
    210                 215                 220

ggc ggt ggg ctt tct gtt cct tat caa cag ggt gaa gag gcg gtt gat      720
Gly Gly Gly Leu Ser Val Pro Tyr Gln Gln Gly Glu Glu Ala Val Asp
225                 230                 235                 240

acc gaa cat tat tat ggt ctg tgg aat gcc gcg cgt gag caa atc gcc      768
Thr Glu His Tyr Tyr Gly Leu Trp Asn Ala Ala Arg Glu Gln Ile Ala
                245                 250                 255

cgc cat ttg ggc cac cct gtg aaa ctg gaa att gaa ccg ggt cgc ttc      816
Arg His Leu Gly His Pro Val Lys Leu Glu Ile Glu Pro Gly Arg Phe
            260                 265                 270

ctg gta gcg cag tct ggc gta tta att act cag gtg cgg agc gtc aaa      864
Leu Val Ala Gln Ser Gly Val Leu Ile Thr Gln Val Arg Ser Val Lys
        275                 280                 285

caa atg ggg agc cgc cac ttt gtg ctg gtt gat gcc ggg ttc aac gat      912
Gln Met Gly Ser Arg His Phe Val Leu Val Asp Ala Gly Phe Asn Asp
    290                 295                 300

ctg atg cgc ccg gca atg tac ggt agt tac cac cat atc agt gcc ctg      960
Leu Met Arg Pro Ala Met Tyr Gly Ser Tyr His His Ile Ser Ala Leu
305                 310                 315                 320

gca gct gat ggt cgt tct ctg gaa cac gcg cca acg gtg gaa acc gtc     1008
Ala Ala Asp Gly Arg Ser Leu Glu His Ala Pro Thr Val Glu Thr Val
                325                 330                 335

gtc gcc gga ccg tta tgt gaa tcg ggc gat gtc ttt acc cag cag gaa     1056
Val Ala Gly Pro Leu Cys Glu Ser Gly Asp Val Phe Thr Gln Gln Glu
            340                 345                 350

ggg gga aat gtt gaa acc cgc gcc ttg ccg gaa gtg aag gca ggt gat     1104
Gly Gly Asn Val Glu Thr Arg Ala Leu Pro Glu Val Lys Ala Gly Asp
        355                 360                 365

tat ctg gta ctg cat gat aca ggg gca tat ggc gca tca atg tca tcc     1152
Tyr Leu Val Leu His Asp Thr Gly Ala Tyr Gly Ala Ser Met Ser Ser
    370                 375                 380

aac tac aat agc cgt ccg ctg tta cca gaa gtt ctg ttt gat aat ggt     1200
Asn Tyr Asn Ser Arg Pro Leu Leu Pro Glu Val Leu Phe Asp Asn Gly
385                 390                 395                 400

cag gcg cgg ttg att cgc cgt cgc cag acc atc gaa gaa tta ctg gcg     1248
Gln Ala Arg Leu Ile Arg Arg Arg Gln Thr Ile Glu Glu Leu Leu Ala
                405                 410                 415

ctg gaa ttg ctt taa                                                1263
Leu Glu Leu Leu
            420
```

<210> 31
<211> 420
<212> PRT
<213> 大腸桿菌

<400> 31

Met Pro His Ser Leu Phe Ser Thr Asp Thr Asp Leu Thr Ala Glu Asn
1 5 10 15

Leu Leu Arg Leu Pro Ala Glu Phe Gly Cys Pro Val Trp Val Tyr Asp
20 25 30

Ala Gln Ile Ile Arg Arg Gln Ile Ala Ala Leu Lys Gln Phe Asp Val
35 40 45

Val Arg Phe Ala Gln Lys Ala Cys Ser Asn Ile His Ile Leu Arg Leu
50 55 60

Met Arg Glu Gln Gly Val Lys Val Asp Ser Val Ser Leu Gly Glu Ile
65 70 75 80

Glu Arg Ala Leu Ala Ala Gly Tyr Asn Pro Gln Thr His Pro Asp Asp
85 90 95

Ile Val Phe Thr Ala Asp Val Ile Asp Gln Ala Thr Leu Glu Arg Val
100 105 110

Ser Glu Leu Gln Ile Pro Val Asn Ala Gly Ser Val Asp Met Leu Asp
115 120 125

Gln Leu Gly Gln Val Ser Pro Gly His Arg Val Trp Leu Arg Val Asn
130 135 140

Pro Gly Phe Gly His Gly His Ser Gln Lys Thr Asn Thr Gly Gly Glu
145 150 155 160

Asn Ser Lys His Gly Ile Trp Tyr Thr Asp Leu Pro Ala Ala Leu Asp
165 170 175

Val Ile Gln Arg His His Leu Gln Leu Val Gly Ile His Met His Ile
180 185 190

Gly Ser Gly Val Asp Tyr Ala His Leu Glu Gln Val Cys Gly Ala Met
195 200 205

Val Arg Gln Val Ile Glu Phe Gly Gln Asp Leu Gln Ala Ile Ser Ala
210 215 220

Gly Gly Gly Leu Ser Val Pro Tyr Gln Gln Gly Glu Glu Ala Val Asp
225 230 235 240

Thr Glu His Tyr Tyr Gly Leu Trp Asn Ala Ala Arg Glu Gln Ile Ala
245 250 255

Arg His Leu Gly His Pro Val Lys Leu Glu Ile Glu Pro Gly Arg Phe
260 265 270

Leu Val Ala Gln Ser Gly Val Leu Ile Thr Gln Val Arg Ser Val Lys
275 280 285

Gln Met Gly Ser Arg His Phe Val Leu Val Asp Ala Gly Phe Asn Asp
290 295 300

Leu Met Arg Pro Ala Met Tyr Gly Ser Tyr His His Ile Ser Ala Leu
305 310 315 320

Ala Ala Asp Gly Arg Ser Leu Glu His Ala Pro Thr Val Glu Thr Val
325 330 335

Val Ala Gly Pro Leu Cys Glu Ser Gly Asp Val Phe Thr Gln Gln Glu
340 345 350

Gly Gly Asn Val Glu Thr Arg Ala Leu Pro Glu Val Lys Ala Gly Asp
355 360 365

Tyr Leu Val Leu His Asp Thr Gly Ala Tyr Gly Ala Ser Met Ser Ser
370 375 380

Asn Tyr Asn Ser Arg Pro Leu Leu Pro Glu Val Leu Phe Asp Asn Gly
385 390 395 400

Gln Ala Arg Leu Ile Arg Arg Arg Gln Thr Ile Glu Glu Leu Leu Ala
405 410 415

Leu Glu Leu Leu
420

<210> 32
<211> 1265
<212> DNA

<213> 人造

<220>
<223> 大腸桿菌二胺庚二酸去羧苷 LysA 密碼子最佳化基因

<400> 32
atatgccaca ctctctgttt tctactgata ctgatctgac tgcggaaaac ctgctgcgtc 60

tgccggctga attcggttgt ccggtatggg tgtacgacgc tcagattatt cgtcgccaga 120

tcgcagcact gaagcagttc gatgtagtgc gttttgcaca gaaggcgtgc tccaacatcc 180

atatcctgcg cctgatgcgt gagcagggcg ttaaagttga ctccgtctct ctgggtgaga 240

ttgagcgcgc cctggcagcc ggctataacc cacagaccca tcctgacgac attgtattta 300

ctgccgacgt gatcgaccag gctactctgg aacgcgtttc tgaactgcag atcccggtta 360

atgctggttc tgtggacatg ctggaccagc tgggccaggt atccccaggt catcgtgtgt 420

ggctgcgtgt caacccaggt ttcggccacg gccactctca gaaaactaac actggtggtg 480

agaactccaa gcatggcatt tggtataccg atctgccggc tgcactggac gtaatccagc 540

gtcaccacct gcagctggtg ggcatccaca tgcacattgg ctccggcgta gactacgccc 600

acctggagca agtctgcggt gctatggtac gtcaggtaat cgagttcggc caagatctgc 660

aggcaatcag cgctggtggc ggcctgtctg taccttatca gcagggcgag gaggcggttg 720

acactgagca ctactacggt ctgtggaacg ccgctcgtga gcaaattgca cgtcacctgg 780

gccacccggt gaaactggag atcgagccgg gccgcttcct ggtagcacag tccggcgtac 840

tgattaccca ggtacgctct gttaaacaga tgggctcccg tcactttgtg ctggtagacg 900

caggcttcaa cgacctgatg cgtccggcta tgtatggttc ctatcatcac atctctgcgc 960

tggccgccga cggccgctct ctggaacacg cgccgacggt tgaaacggtg gtggctggtc 1020

cgctgtgcga gtccggcgac gttttcactc agcaggaggg cggcaatgta gagacgcgtg 1080

cgctgccgga agtgaaagcc ggtgattatc tggtgctgca tgataccggc gcctatggtg 1140

cgagcatgag cagcaactac aactctcgcc cgctgctgcc ggaggtcctg ttcgataacg 1200

gccaagcccg cctgatccgt cgtcgtcaga ccatcgagga actgctggca ctggagctgc 1260

tgtaa 1265

<210> 33
<211> 1692
<212> DNA
<213> 釀酒酵母

<220>
<221> CDS

<222> (1)..(1692)

<400> 33

```
atg tct gaa att act ttg ggt aaa tat ttg ttc gaa aga tta aag caa      48
Met Ser Glu Ile Thr Leu Gly Lys Tyr Leu Phe Glu Arg Leu Lys Gln
1               5                  10                  15

gtc aac gtt aac acc gtt ttc ggt ttg cca ggt gac ttc aac ttg tcc      96
Val Asn Val Asn Thr Val Phe Gly Leu Pro Gly Asp Phe Asn Leu Ser
            20                  25                  30

ttg ttg gac aag atc tac gaa gtt gaa ggt atg aga tgg gct ggt aac     144
Leu Leu Asp Lys Ile Tyr Glu Val Glu Gly Met Arg Trp Ala Gly Asn
        35                  40                  45

gcc aac gaa ttg aac gct gct tac gcc gct gat ggt tac gct cgt atc     192
Ala Asn Glu Leu Asn Ala Ala Tyr Ala Ala Asp Gly Tyr Ala Arg Ile
    50                  55                  60

aag ggt atg tct tgt atc atc acc acc ttc ggt gtc ggt gaa ttg tct     240
Lys Gly Met Ser Cys Ile Ile Thr Thr Phe Gly Val Gly Glu Leu Ser
65                  70                  75                  80

gct ttg aac ggt att gcc ggt tct tac gct gaa cac gtc ggt gtt ttg     288
Ala Leu Asn Gly Ile Ala Gly Ser Tyr Ala Glu His Val Gly Val Leu
                85                  90                  95

cac gtt gtt ggt gtc cca tcc atc tct gct caa gct aag caa ttg ttg     336
His Val Val Gly Val Pro Ser Ile Ser Ala Gln Ala Lys Gln Leu Leu
            100                 105                 110

ttg cac cac acc ttg ggt aac ggt gac ttc act gtt ttc cac aga atg     384
Leu His His Thr Leu Gly Asn Gly Asp Phe Thr Val Phe His Arg Met
        115                 120                 125

tct gcc aac att tct gaa acc act gct atg atc act gac att gct acc     432
Ser Ala Asn Ile Ser Glu Thr Thr Ala Met Ile Thr Asp Ile Ala Thr
    130                 135                 140

gcc cca gct gaa att gac aga tgt atc aga acc act tac gtc acc caa     480
Ala Pro Ala Glu Ile Asp Arg Cys Ile Arg Thr Thr Tyr Val Thr Gln
145                 150                 155                 160

aga cca gtc tac tta ggt ttg cca gct aac ttg gtc gac ttg aac gtc     528
Arg Pro Val Tyr Leu Gly Leu Pro Ala Asn Leu Val Asp Leu Asn Val
                165                 170                 175

cca gct aag ttg ttg caa act cca att gac atg tct ttg aag cca aac     576
Pro Ala Lys Leu Leu Gln Thr Pro Ile Asp Met Ser Leu Lys Pro Asn
            180                 185                 190

gat gct gaa tcc gaa aag gaa gtc att gac acc atc ttg gct ttg gtc     624
Asp Ala Glu Ser Glu Lys Glu Val Ile Asp Thr Ile Leu Ala Leu Val
        195                 200                 205

aag gat gct aag aac cca gtt atc ttg gct gat gct tgt tgt tcc aga     672
Lys Asp Ala Lys Asn Pro Val Ile Leu Ala Asp Ala Cys Cys Ser Arg
    210                 215                 220
```

```
cac gac gtc aag gct gaa act aag aag ttg att gac ttg act caa ttc      720
His Asp Val Lys Ala Glu Thr Lys Lys Leu Ile Asp Leu Thr Gln Phe
225                 230                 235                 240

cca gct ttc gtc acc cca atg ggt aag ggt tcc att gac gaa caa cac      768
Pro Ala Phe Val Thr Pro Met Gly Lys Gly Ser Ile Asp Glu Gln His
                245                 250                 255

cca aga tac ggt ggt gtt tac gtc ggt acc ttg tcc aag cca gaa gtt      816
Pro Arg Tyr Gly Gly Val Tyr Val Gly Thr Leu Ser Lys Pro Glu Val
            260                 265                 270

aag gaa gcc gtt gaa tct gct gac ttg att ttg tct gtc ggt gct ttg      864
Lys Glu Ala Val Glu Ser Ala Asp Leu Ile Leu Ser Val Gly Ala Leu
        275                 280                 285

ttg tct gat ttc aac acc ggt tct ttc tct tac tct tac aag acc aag      912
Leu Ser Asp Phe Asn Thr Gly Ser Phe Ser Tyr Ser Tyr Lys Thr Lys
    290                 295                 300

aac att gtc gaa ttc cac tcc gac cac atg aag atc aga aac gcc act      960
Asn Ile Val Glu Phe His Ser Asp His Met Lys Ile Arg Asn Ala Thr
305                 310                 315                 320

ttc cca ggt gtc caa atg aaa ttc gtt ttg caa aag ttg ttg acc act     1008
Phe Pro Gly Val Gln Met Lys Phe Val Leu Gln Lys Leu Leu Thr Thr
                325                 330                 335

att gct gac gcc gct aag ggt tac aag cca gtt gct gtc cca gct aga     1056
Ile Ala Asp Ala Ala Lys Gly Tyr Lys Pro Val Ala Val Pro Ala Arg
            340                 345                 350

act cca gct aac gct gct gtc cca gct tct acc cca ttg aag caa gaa     1104
Thr Pro Ala Asn Ala Ala Val Pro Ala Ser Thr Pro Leu Lys Gln Glu
        355                 360                 365

tgg atg tgg aac caa ttg ggt aac ttc ttg caa gaa ggt gat gtt gtc     1152
Trp Met Trp Asn Gln Leu Gly Asn Phe Leu Gln Glu Gly Asp Val Val
    370                 375                 380

att gct gaa acc ggt acc tcc gct ttc ggt atc aac caa acc act ttc     1200
Ile Ala Glu Thr Gly Thr Ser Ala Phe Gly Ile Asn Gln Thr Thr Phe
385                 390                 395                 400

cca aac aac acc tac ggt atc tct caa gtc tta tgg ggt tcc att ggt     1248
Pro Asn Asn Thr Tyr Gly Ile Ser Gln Val Leu Trp Gly Ser Ile Gly
                405                 410                 415

ttc acc act ggt gct acc ttg ggt gct gct ttc gct gct gaa gaa att     1296
Phe Thr Thr Gly Ala Thr Leu Gly Ala Ala Phe Ala Ala Glu Glu Ile
            420                 425                 430

gat cca aag aag aga gtt atc tta ttc att ggt gac ggt tct ttg caa     1344
Asp Pro Lys Lys Arg Val Ile Leu Phe Ile Gly Asp Gly Ser Leu Gln
        435                 440                 445

ttg act gtt caa gaa atc tcc acc atg atc aga tgg ggc ttg aag cca     1392
Leu Thr Val Gln Glu Ile Ser Thr Met Ile Arg Trp Gly Leu Lys Pro
    450                 455                 460
```

```
tac ttg ttc gtc ttg aac aac gat ggt tac acc att gaa aag ttg att      1440
Tyr Leu Phe Val Leu Asn Asn Asp Gly Tyr Thr Ile Glu Lys Leu Ile
465                 470                 475                 480

cac ggt cca aag gct caa tac aac gaa att caa ggt tgg gac cac cta      1488
His Gly Pro Lys Ala Gln Tyr Asn Glu Ile Gln Gly Trp Asp His Leu
                485                 490                 495

tcc ttg ttg cca act ttc ggt gct aag gac tat gaa acc cac aga gtc      1536
Ser Leu Leu Pro Thr Phe Gly Ala Lys Asp Tyr Glu Thr His Arg Val
            500                 505                 510

gct acc acc ggt gaa tgg gac aag ttg acc caa gac aag tct ttc aac      1584
Ala Thr Thr Gly Glu Trp Asp Lys Leu Thr Gln Asp Lys Ser Phe Asn
        515                 520                 525

gac aac tct aag atc aga atg att gaa atc atg ttg cca gtc ttc gat      1632
Asp Asn Ser Lys Ile Arg Met Ile Glu Ile Met Leu Pro Val Phe Asp
    530                 535                 540

gct cca caa aac ttg gtt gaa caa gct aag ttg act gct gct acc aac      1680
Ala Pro Gln Asn Leu Val Glu Gln Ala Lys Leu Thr Ala Ala Thr Asn
545                 550                 555                 560

gct aag caa taa                                                     1692
Ala Lys Gln
```

<210> 34
<211> 563
<212> PRT
<213> 釀酒酵母

<400> 34

```
Met Ser Glu Ile Thr Leu Gly Lys Tyr Leu Phe Glu Arg Leu Lys Gln
1               5                   10                  15

Val Asn Val Asn Thr Val Phe Gly Leu Pro Gly Asp Phe Asn Leu Ser
            20                  25                  30

Leu Leu Asp Lys Ile Tyr Glu Val Glu Gly Met Arg Trp Ala Gly Asn
        35                  40                  45

Ala Asn Glu Leu Asn Ala Ala Tyr Ala Ala Asp Gly Tyr Ala Arg Ile
    50                  55                  60

Lys Gly Met Ser Cys Ile Ile Thr Thr Phe Gly Val Gly Glu Leu Ser
65                  70                  75                  80

Ala Leu Asn Gly Ile Ala Gly Ser Tyr Ala Glu His Val Gly Val Leu
                85                  90                  95
```

His Val Val Gly Val Pro Ser Ile Ser Ala Gln Ala Lys Gln Leu Leu
100 105 110

Leu His His Thr Leu Gly Asn Gly Asp Phe Thr Val Phe His Arg Met
115 120 125

Ser Ala Asn Ile Ser Glu Thr Thr Ala Met Ile Thr Asp Ile Ala Thr
130 135 140

Ala Pro Ala Glu Ile Asp Arg Cys Ile Arg Thr Thr Tyr Val Thr Gln
145 150 155 160

Arg Pro Val Tyr Leu Gly Leu Pro Ala Asn Leu Val Asp Leu Asn Val
165 170 175

Pro Ala Lys Leu Leu Gln Thr Pro Ile Asp Met Ser Leu Lys Pro Asn
180 185 190

Asp Ala Glu Ser Glu Lys Glu Val Ile Asp Thr Ile Leu Ala Leu Val
195 200 205

Lys Asp Ala Lys Asn Pro Val Ile Leu Ala Asp Ala Cys Cys Ser Arg
210 215 220

His Asp Val Lys Ala Glu Thr Lys Lys Leu Ile Asp Leu Thr Gln Phe
225 230 235 240

Pro Ala Phe Val Thr Pro Met Gly Lys Gly Ser Ile Asp Glu Gln His
245 250 255

Pro Arg Tyr Gly Gly Val Tyr Val Gly Thr Leu Ser Lys Pro Glu Val
260 265 270

Lys Glu Ala Val Glu Ser Ala Asp Leu Ile Leu Ser Val Gly Ala Leu
275 280 285

Leu Ser Asp Phe Asn Thr Gly Ser Phe Ser Tyr Ser Tyr Lys Thr Lys
290 295 300

Asn Ile Val Glu Phe His Ser Asp His Met Lys Ile Arg Asn Ala Thr
305 310 315 320

Phe Pro Gly Val Gln Met Lys Phe Val Leu Gln Lys Leu Leu Thr Thr

325 330 335

Ile Ala Asp Ala Ala Lys Gly Tyr Lys Pro Val Ala Val Pro Ala Arg
340 345 350

Thr Pro Ala Asn Ala Ala Val Pro Ala Ser Thr Pro Leu Lys Gln Glu
355 360 365

Trp Met Trp Asn Gln Leu Gly Asn Phe Leu Gln Glu Gly Asp Val Val
370 375 380

Ile Ala Glu Thr Gly Thr Ser Ala Phe Gly Ile Asn Gln Thr Thr Phe
385 390 395 400

Pro Asn Asn Thr Tyr Gly Ile Ser Gln Val Leu Trp Gly Ser Ile Gly
405 410 415

Phe Thr Thr Gly Ala Thr Leu Gly Ala Ala Phe Ala Ala Glu Glu Ile
420 425 430

Asp Pro Lys Lys Arg Val Ile Leu Phe Ile Gly Asp Gly Ser Leu Gln
435 440 445

Leu Thr Val Gln Glu Ile Ser Thr Met Ile Arg Trp Gly Leu Lys Pro
450 455 460

Tyr Leu Phe Val Leu Asn Asn Asp Gly Tyr Thr Ile Glu Lys Leu Ile
465 470 475 480

His Gly Pro Lys Ala Gln Tyr Asn Glu Ile Gln Gly Trp Asp His Leu
485 490 495

Ser Leu Leu Pro Thr Phe Gly Ala Lys Asp Tyr Glu Thr His Arg Val
500 505 510

Ala Thr Thr Gly Glu Trp Asp Lys Leu Thr Gln Asp Lys Ser Phe Asn
515 520 525

Asp Asn Ser Lys Ile Arg Met Ile Glu Ile Met Leu Pro Val Phe Asp
530 535 540

Ala Pro Gln Asn Leu Val Glu Gln Ala Lys Leu Thr Ala Ala Thr Asn
545 550 555 560

Ala Lys Gln

<210> 35
<211> 1692
<212> DNA
<213> 人造

<220>
<223> 釀酒酵母丙酮酸去羧苷 Pdc 密碼子最佳化基因

<400> 35
atgtccgaga tcactctggg caaatacctg tttgaacgtc tgaaacaggt gaacgttaat 60

accgtattcg gcctgccggg tgatttcaac ctgtccctgc tggacaaaat ctatgaagtt 120

gaaggtatgc gttgggctgg caacgctaac gagctgaacg cagcgtacgc ggcagatggt 180

tacgctcgta tcaaaggtat gtcttgtatc atcaccacct tcggtgttgg tgagctgagc 240

gccctgaacg gcatcgccgg ctcctatgca gagcacgtgg gcgtgctgca cgttgtgggt 300

gtaccgtcca tcagcgccca ggcaaaacag ctgctgctgc accacaccct gggtaacggc 360

gactttaccg ttttccatcg tatgtctgcg aacatcagcg aaactactgc aatgattact 420

gacatcgcta cggcaccggc agaaatcgac cgttgcattc gtaccacgta cgttactcag 480

cgcccggttt atctgggcct gccagccaac ctggtggatc tgaacgtccc ggctaaactg 540

ctgcagactc cgatcgatat gtctctgaaa cctaacgacg cagaatctga gaaagaagtt 600

atcgatacta ttctggctct ggtgaaagat gcaaagaacc cagttatcct ggctgacgca 660

tgttgctctc gtcatgatgt aaaggcagaa accaaaaagc tgatcgacct gacgcagttc 720

ccggcgttcg ttaccccgat gggcaagggt tccatcgatg agcagcaccc gcgttatggt 780

ggtgtatacg ttggcacgct gtccaaaccg gaggtaaaag aagcggttga aagcgcagat 840

ctgatcctgt ctgttggtgc actgctgagc gacttcaaca ccggttcttt ctcctatagc 900

tacaagacca aaaacattgt ggagtttcac tccgatcaca tgaaaatccg caacgcgacc 960

tttcctggtg tgcagatgaa attcgtactg cagaaactgc tgaccaccat cgccgacgct 1020

gcgaaaggtt ataaaccggt agctgtgccg gcacgtaccc cggcgaacgc cgcggttcct 1080

gcatccactc cactgaagca ggaatggatg tggaatcagc tgggtaattt cctgcaagaa 1140

ggcgacgttg taatcgcaga aaccggcact agcgcgtttg gcattaacca gacgaccttc 1200

ccaaacaaca cctacggtat cagccaagtc ctgtggggct ctatcggctt caccaccggt 1260

gcaaccctgg gtgcggcttt cgctgctgag gagatcgacc cgaagaaacg tgttatcctg 1320

ttcatcggtg acggctccct gcagctgacc gtccaggaga tttctaccat gatccgctgg 1380

ggcctgaaac cgtacctgtt tgtgctgaac aacgacggct acactattga gaaactgatc 1440

cacggtccga aagcacagta taatgagatc cagggttggg atcatctgtc tctgctgccg 1500

acctttggcg ctaaagacta cgagacccac cgcgtggcta ccaccggcga gtgggataaa 1560

ctgacgcagg ataaatcctt caatgacaat agcaagattc gtatgatcga aatcatgctg 1620

ccggtctttg atgctccgca gaacctggta gagcaagcaa aactgaccgc ggcaactaac 1680

gctaaacagt aa 1692

<210> 36
<211> 1707
<212> DNA
<213> 活動發酵單胞菌

<220>
<221> CDS
<222> (1)..(1707)

<400> 36

```
atg agt tat act gtc ggt acc tat tta gcg gag cgg ctt gtc cag att      48
Met Ser Tyr Thr Val Gly Thr Tyr Leu Ala Glu Arg Leu Val Gln Ile
1               5                  10                  15

ggt ctc aag cat cac ttc gca gtc gcg ggc gac tac aac ctc gtc ctt      96
Gly Leu Lys His His Phe Ala Val Ala Gly Asp Tyr Asn Leu Val Leu
            20                  25                  30

ctt gac aac ctg ctt ttg aac aaa aac atg gag cag gtt tat tgc tgt     144
Leu Asp Asn Leu Leu Leu Asn Lys Asn Met Glu Gln Val Tyr Cys Cys
        35                  40                  45

aac gaa ctg aac tgc ggt ttc agt gca gaa ggt tat gct cgt gcc aaa     192
Asn Glu Leu Asn Cys Gly Phe Ser Ala Glu Gly Tyr Ala Arg Ala Lys
    50                  55                  60

ggc gca gca gca gcc gtc gtt acc tac agc gtc ggt gcg ctt tcc gca     240
Gly Ala Ala Ala Ala Val Val Thr Tyr Ser Val Gly Ala Leu Ser Ala
65                  70                  75                  80

ttt gat gct atc ggt ggc gcc tat gca gaa aac ctt ccg gtt atc ctg     288
Phe Asp Ala Ile Gly Gly Ala Tyr Ala Glu Asn Leu Pro Val Ile Leu
                85                  90                  95

atc tcc ggt gct ccg aac aac aat gat cac gct gct ggt cac gtg ttg     336
Ile Ser Gly Ala Pro Asn Asn Asn Asp His Ala Ala Gly His Val Leu
            100                 105                 110

cat cac gct ctt ggc aaa acc gac tat cac tat cag ttg gaa atg gcc     384
His His Ala Leu Gly Lys Thr Asp Tyr His Tyr Gln Leu Glu Met Ala
        115                 120                 125

aag aac atc acg gcc gcc gct gaa gcg att tac acc ccg gaa gaa gct     432
Lys Asn Ile Thr Ala Ala Ala Glu Ala Ile Tyr Thr Pro Glu Glu Ala
    130                 135                 140
```

```
ccg gct aaa atc gat cac gtg att aaa act gct ctt cgt gag aag aag      480
Pro Ala Lys Ile Asp His Val Ile Lys Thr Ala Leu Arg Glu Lys Lys
145                 150                 155                 160

ccg gtt tat ctc gaa atc gct tgc aac att gct tcc atg ccc tgc gcc      528
Pro Val Tyr Leu Glu Ile Ala Cys Asn Ile Ala Ser Met Pro Cys Ala
                165                 170                 175

gct cct gga ccg gca agc gca ttg ttc aat gac gaa gcc agc gac gaa      576
Ala Pro Gly Pro Ala Ser Ala Leu Phe Asn Asp Glu Ala Ser Asp Glu
            180                 185                 190

gct tct ttg aat gca gcg gtt gaa gaa acc ctg aaa ttc atc gcc aac      624
Ala Ser Leu Asn Ala Ala Val Glu Glu Thr Leu Lys Phe Ile Ala Asn
        195                 200                 205

cgc gac aaa gtt gcc gtc ctc gtc ggc agc aag ctg cgc gca gct ggt      672
Arg Asp Lys Val Ala Val Leu Val Gly Ser Lys Leu Arg Ala Ala Gly
    210                 215                 220

gct gaa gaa gct gct gtc aaa ttt gct gat gct ctc ggt ggc gca gtt      720
Ala Glu Glu Ala Ala Val Lys Phe Ala Asp Ala Leu Gly Gly Ala Val
225                 230                 235                 240

gct acc atg gct gct gca aaa agc ttc ttc cca gaa gaa aac ccg cat      768
Ala Thr Met Ala Ala Ala Lys Ser Phe Phe Pro Glu Glu Asn Pro His
                245                 250                 255

tac atc ggc acc tca tgg ggt gaa gtc agc tat ccg ggc gtt gaa aag      816
Tyr Ile Gly Thr Ser Trp Gly Glu Val Ser Tyr Pro Gly Val Glu Lys
            260                 265                 270

acg atg aaa gaa gcc gat gcg gtt atc gct ctg gct cct gtc ttc aac      864
Thr Met Lys Glu Ala Asp Ala Val Ile Ala Leu Ala Pro Val Phe Asn
        275                 280                 285

gac tac tcc acc act ggt tgg acg gat att cct gat cct aag aaa ctg      912
Asp Tyr Ser Thr Thr Gly Trp Thr Asp Ile Pro Asp Pro Lys Lys Leu
    290                 295                 300

gtt ctc gct gaa ccg cgt tct gtc gtc gtt aac ggc att cgc ttc ccc      960
Val Leu Ala Glu Pro Arg Ser Val Val Val Asn Gly Ile Arg Phe Pro
305                 310                 315                 320

agc gtc cat ctg aaa gac tat ctg acc cgt ttg gct cag aaa gtt tcc     1008
Ser Val His Leu Lys Asp Tyr Leu Thr Arg Leu Ala Gln Lys Val Ser
                325                 330                 335

aag aaa acc ggt gca ttg gac ttc ttc aaa tcc ctc aat gca ggt gaa     1056
Lys Lys Thr Gly Ala Leu Asp Phe Phe Lys Ser Leu Asn Ala Gly Glu
            340                 345                 350

ctg aag aaa gcc gct ccg gct gat ccg agt gct ccg ttg gtc aac gca     1104
Leu Lys Lys Ala Ala Pro Ala Asp Pro Ser Ala Pro Leu Val Asn Ala
        355                 360                 365

gaa atc gcc cgt cag gtc gaa gct ctt ctg acc ccg aac acg acg gtt     1152
Glu Ile Ala Arg Gln Val Glu Ala Leu Leu Thr Pro Asn Thr Thr Val
```

370 375 380

att gct gaa acc ggt gac tct tgg ttc aat gct cag cgc atg aag ctc 1200
Ile Ala Glu Thr Gly Asp Ser Trp Phe Asn Ala Gln Arg Met Lys Leu
385 390 395 400

ccg aac ggt gct cgc gtt gaa tat gaa atg cag tgg ggt cac att ggt 1248
Pro Asn Gly Ala Arg Val Glu Tyr Glu Met Gln Trp Gly His Ile Gly
405 410 415

tgg tcc gtt cct gcc gcc ttc ggt tat gcc gtc ggt gct ccg gaa cgt 1296
Trp Ser Val Pro Ala Ala Phe Gly Tyr Ala Val Gly Ala Pro Glu Arg
420 425 430

cgc aac atc ctc atg gtt ggt gat ggt tcc ttc cag ctg acg gct cag 1344
Arg Asn Ile Leu Met Val Gly Asp Gly Ser Phe Gln Leu Thr Ala Gln
435 440 445

gaa gtc gct cag atg gtt cgc ctg aaa ctg ccg gtt atc atc ttc ttg 1392
Glu Val Ala Gln Met Val Arg Leu Lys Leu Pro Val Ile Ile Phe Leu
450 455 460

atc aat aac tat ggt tac acc gcc gaa gtt atg atc cat gat ggt ccg 1440
Ile Asn Asn Tyr Gly Tyr Thr Ala Glu Val Met Ile His Asp Gly Pro
465 470 475 480

tac aac aac atc aag aac tgg gat tat gcc ggt ctg atg gaa gtg ttc 1488
Tyr Asn Asn Ile Lys Asn Trp Asp Tyr Ala Gly Leu Met Glu Val Phe
485 490 495

aac ggt aac ggt ggt tat gac agc ggt gct ggt aaa ggc ctg aag gct 1536
Asn Gly Asn Gly Gly Tyr Asp Ser Gly Ala Gly Lys Gly Leu Lys Ala
500 505 510

aaa acc ggt ggc gaa ctg gca gaa gct atc aag gtt gct ctg gca aac 1584
Lys Thr Gly Gly Glu Leu Ala Glu Ala Ile Lys Val Ala Leu Ala Asn
515 520 525

acc gac ggc cca acc ctg atc gaa tgc ttc atc ggt cgt gaa gac tgc 1632
Thr Asp Gly Pro Thr Leu Ile Glu Cys Phe Ile Gly Arg Glu Asp Cys
530 535 540

act gaa gaa ttg gtc aaa tgg ggt aag cgc gtt gct gcc gcc aac agc 1680
Thr Glu Glu Leu Val Lys Trp Gly Lys Arg Val Ala Ala Ala Asn Ser
545 550 555 560

cgt aag cct gtt aac aag ctc ctc tag 1707
Arg Lys Pro Val Asn Lys Leu Leu
565

<210> 37
<211> 568
<212> PRT
<213> 活動發酵單胞菌

<400> 37

Met Ser Tyr Thr Val Gly Thr Tyr Leu Ala Glu Arg Leu Val Gln Ile

1 5 10 15

Gly Leu Lys His His Phe Ala Val Ala Gly Asp Tyr Asn Leu Val Leu
20 25 30

Leu Asp Asn Leu Leu Leu Asn Lys Asn Met Glu Gln Val Tyr Cys Cys
35 40 45

Asn Glu Leu Asn Cys Gly Phe Ser Ala Glu Gly Tyr Ala Arg Ala Lys
50 55 60

Gly Ala Ala Ala Ala Val Val Thr Tyr Ser Val Gly Ala Leu Ser Ala
65 70 75 80

Phe Asp Ala Ile Gly Gly Ala Tyr Ala Glu Asn Leu Pro Val Ile Leu
85 90 95

Ile Ser Gly Ala Pro Asn Asn Asn Asp His Ala Ala Gly His Val Leu
100 105 110

His His Ala Leu Gly Lys Thr Asp Tyr His Tyr Gln Leu Glu Met Ala
115 120 125

Lys Asn Ile Thr Ala Ala Ala Glu Ala Ile Tyr Thr Pro Glu Glu Ala
130 135 140

Pro Ala Lys Ile Asp His Val Ile Lys Thr Ala Leu Arg Glu Lys Lys
145 150 155 160

Pro Val Tyr Leu Glu Ile Ala Cys Asn Ile Ala Ser Met Pro Cys Ala
165 170 175

Ala Pro Gly Pro Ala Ser Ala Leu Phe Asn Asp Glu Ala Ser Asp Glu
180 185 190

Ala Ser Leu Asn Ala Ala Val Glu Glu Thr Leu Lys Phe Ile Ala Asn
195 200 205

Arg Asp Lys Val Ala Val Leu Val Gly Ser Lys Leu Arg Ala Ala Gly
210 215 220

Ala Glu Glu Ala Ala Val Lys Phe Ala Asp Ala Leu Gly Gly Ala Val
225 230 235 240

Ala Thr Met Ala Ala Ala Lys Ser Phe Phe Pro Glu Glu Asn Pro His
245 250 255

Tyr Ile Gly Thr Ser Trp Gly Glu Val Ser Tyr Pro Gly Val Glu Lys
260 265 270

Thr Met Lys Glu Ala Asp Ala Val Ile Ala Leu Ala Pro Val Phe Asn
275 280 285

Asp Tyr Ser Thr Thr Gly Trp Thr Asp Ile Pro Asp Pro Lys Lys Leu
290 295 300

Val Leu Ala Glu Pro Arg Ser Val Val Val Asn Gly Ile Arg Phe Pro
305 310 315 320

Ser Val His Leu Lys Asp Tyr Leu Thr Arg Leu Ala Gln Lys Val Ser
325 330 335

Lys Lys Thr Gly Ala Leu Asp Phe Phe Lys Ser Leu Asn Ala Gly Glu
340 345 350

Leu Lys Lys Ala Ala Pro Ala Asp Pro Ser Ala Pro Leu Val Asn Ala
355 360 365

Glu Ile Ala Arg Gln Val Glu Ala Leu Leu Thr Pro Asn Thr Thr Val
370 375 380

Ile Ala Glu Thr Gly Asp Ser Trp Phe Asn Ala Gln Arg Met Lys Leu
385 390 395 400

Pro Asn Gly Ala Arg Val Glu Tyr Glu Met Gln Trp Gly His Ile Gly
405 410 415

Trp Ser Val Pro Ala Ala Phe Gly Tyr Ala Val Gly Ala Pro Glu Arg
420 425 430

Arg Asn Ile Leu Met Val Gly Asp Gly Ser Phe Gln Leu Thr Ala Gln
435 440 445

Glu Val Ala Gln Met Val Arg Leu Lys Leu Pro Val Ile Ile Phe Leu
450 455 460

Ile Asn Asn Tyr Gly Tyr Thr Ala Glu Val Met Ile His Asp Gly Pro
465 470 475 480

Tyr Asn Asn Ile Lys Asn Trp Asp Tyr Ala Gly Leu Met Glu Val Phe
485 490 495

Asn Gly Asn Gly Gly Tyr Asp Ser Gly Ala Gly Lys Gly Leu Lys Ala
500 505 510

Lys Thr Gly Gly Glu Leu Ala Glu Ala Ile Lys Val Ala Leu Ala Asn
515 520 525

Thr Asp Gly Pro Thr Leu Ile Glu Cys Phe Ile Gly Arg Glu Asp Cys
530 535 540

Thr Glu Glu Leu Val Lys Trp Gly Lys Arg Val Ala Ala Ala Asn Ser
545 550 555 560

Arg Lys Pro Val Asn Lys Leu Leu
565

<210> 38
<211> 1707
<212> DNA
<213> 人造

<220>
<223> 活動發酵單胞菌丙酮酸去羧苷 PdcI472A 密碼子最佳化基因

<400> 38
atgtcttata ctgttggtac ttatctggct gagcgtctgg tgcaaatcgg cctgaaacac 60

cactttgcag ttgctggcga ctacaacctg gttctgctgg ataacctgct gctgaacaaa 120

aacatggagc aagtttattg ctgtaacgag ctgaactgcg gcttctctgc ggagggttat 180

gcgcgtgcga aaggtgccgc tgcagcagtc gtaacctact ctgtgggcgc tctgtccgcg 240

ttcgacgcaa tcggtggcgc ttacgctgaa aacctgccgg tgatcctgat tagcggtgcg 300

ccgaataata acgaccatgc tgctggccac gttctgcacc acgccctggg taaaactgat 360

taccattacc agctggagat ggctaaaaac atcactgcag cagcagaagc gatctacacc 420

ccggaagagg ctccggcaaa aatcgaccac gtgattaaaa ccgctctgcg tgagaaaaag 480

ccggtatacc tggaaatcgc gtgcaacatc gcgtctatgc cgtgcgccgc accgggtccg 540

gcttctgccc tgttcaacga tgaggcgagc gatgaggcat ctctgaacgc agcagtagaa 600

gaaaccctga aatttatcgc aaaccgtgac aaagtagcag tcctggtagg ttctaaactg 660

cgtgcggctg gtgcggaaga ggctgcggta aagttcgcgg atgctctggg cggtgcagtg 720

gcgaccatgg cagcggctaa atccttcttc ccagaggaga acccgcatta cattggtacc 780

tcctggggcg aagtttccta ccctggtgtg gagaaaacca tgaaagaagc cgatgctgtg 840

attgccctgg cgcctgtatt caacgattat tccaccaccg gttggaccga tatcccggac 900

ccgaagaaac tggtcctggc tgaaccgcgc tccgtagtag tgaatggcat tcgtttcccg 960

tccgtacacc tgaaggatta cctgacgcgt ctggcacaga aagtatccaa gaaaactggc 1020

gcgctggact tctttaaatc cctgaacgct ggtgagctga aaaaggcggc tccggccgat 1080

ccgtccgcac cgctggtgaa cgcagagatt gcacgtcagg ttgaggcact gctgacgccg 1140

aacaccaccg taatcgcgga aacgggcgac tcttggttca acgcacagcg catgaaactg 1200

ccgaacggtg cccgcgttga atatgaaatg cagtggggtc acatcggctg gtctgtccca 1260

gcagcgtttg gttacgcggt tggtgcaccg gagcgtcgca acatcctgat ggtgggtgac 1320

ggctccttcc agctgactgc tcaggaggtg gcgcagatgg tgcgcctgaa gctgccggtt 1380

atcattttcc tgatcaacaa ctacggctac accgccgagg taatgatcca cgatggtccg 1440

tacaacaaca tcaaaaactg ggactacgcc ggtctgatgg aggttttaa cggtaacggc 1500

ggttacgaca gcggtgctgg taagggtctg aaagccaaaa ccggtggcga actggcagag 1560

gcgattaaag ttgcgctggc aaacaccgat ggcccgaccc tgatcgagtg cttcatcggc 1620

cgtgaggact gcaccgagga gctggtcaaa tggggcaaac gtgtggcggc tgctaactct 1680

cgcaagccgg taaacaaact gctgtaa 1707

<210> 39
<211> 1644
<212> DNA
<213> 乳酸乳球菌

<220>
<221> CDS
<222> (1)..(1644)

<400> 39

```
atg tat aca gta gga gat tac ctg tta gac cga tta cac gag ttg gga      48
Met Tyr Thr Val Gly Asp Tyr Leu Leu Asp Arg Leu His Glu Leu Gly
1               5                  10                  15

att gaa gaa att ttt gga gtt cct ggt gac tat aac tta caa ttt tta      96
Ile Glu Glu Ile Phe Gly Val Pro Gly Asp Tyr Asn Leu Gln Phe Leu
            20                  25                  30

gat caa att att tca cgc gaa gat atg aaa tgg att gga aat gct aat     144
Asp Gln Ile Ile Ser Arg Glu Asp Met Lys Trp Ile Gly Asn Ala Asn
        35                  40                  45

gaa tta aat gct tct tat atg gct gat ggt tat gct cgt act aaa aaa     192
Glu Leu Asn Ala Ser Tyr Met Ala Asp Gly Tyr Ala Arg Thr Lys Lys
```

```
    50                  55                  60

gct gcc gca ttt ctc acc aca ttt gga gtc ggc gaa ttg agt gcg atc      240
Ala Ala Ala Phe Leu Thr Thr Phe Gly Val Gly Glu Leu Ser Ala Ile
65                  70                  75                  80

aat gga ctg gca gga agt tat gcc gaa aat tta cca gta gta gaa att      288
Asn Gly Leu Ala Gly Ser Tyr Ala Glu Asn Leu Pro Val Val Glu Ile
                85                  90                  95

gtt ggt tca cca act tca aaa gta caa aat gac gga aaa ttt gtc cat      336
Val Gly Ser Pro Thr Ser Lys Val Gln Asn Asp Gly Lys Phe Val His
            100                 105                 110

cat aca cta gca gat ggt gat ttt aaa cac ttt atg aag atg cat gaa      384
His Thr Leu Ala Asp Gly Asp Phe Lys His Phe Met Lys Met His Glu
        115                 120                 125

cct gtt aca gca gcg cgg act tta ctg aca gca gaa aat gcc aca tat      432
Pro Val Thr Ala Ala Arg Thr Leu Leu Thr Ala Glu Asn Ala Thr Tyr
    130                 135                 140

gaa att gac cga gta ctt tct caa tta cta aaa gaa aga aaa cca gtc      480
Glu Ile Asp Arg Val Leu Ser Gln Leu Leu Lys Glu Arg Lys Pro Val
145                 150                 155                 160

tat att aac tta cca gtc gat gtt gct gca gca aaa gca gag aag cct      528
Tyr Ile Asn Leu Pro Val Asp Val Ala Ala Ala Lys Ala Glu Lys Pro
                165                 170                 175

gca tta tct tta gaa aaa gaa agc tct aca aca aat aca act gaa caa      576
Ala Leu Ser Leu Glu Lys Glu Ser Ser Thr Thr Asn Thr Thr Glu Gln
            180                 185                 190

gtg att ttg agt aag att gaa gaa agt ttg aaa aat gcc caa aaa cca      624
Val Ile Leu Ser Lys Ile Glu Glu Ser Leu Lys Asn Ala Gln Lys Pro
        195                 200                 205

gta gtg att gca gga cac gaa gta att agt ttt ggt tta gaa aaa acg      672
Val Val Ile Ala Gly His Glu Val Ile Ser Phe Gly Leu Glu Lys Thr
    210                 215                 220

gta act cag ttt gtt tca gaa aca aaa cta ccg att acg aca cta aat      720
Val Thr Gln Phe Val Ser Glu Thr Lys Leu Pro Ile Thr Thr Leu Asn
225                 230                 235                 240

ttt ggt aaa agt gct gtt gat gaa tct ttg ccc tca ttt tta gga ata      768
Phe Gly Lys Ser Ala Val Asp Glu Ser Leu Pro Ser Phe Leu Gly Ile
                245                 250                 255

tat aac ggg aaa ctt tca gaa atc agt ctt aaa aat ttt gtg gag tcc      816
Tyr Asn Gly Lys Leu Ser Glu Ile Ser Leu Lys Asn Phe Val Glu Ser
            260                 265                 270

gca gac ttt atc cta atg ctt gga gtg aag ctt acg gac tcc tca aca      864
Ala Asp Phe Ile Leu Met Leu Gly Val Lys Leu Thr Asp Ser Ser Thr
        275                 280                 285

ggt gca ttc aca cat cat tta gat gaa aat aaa atg att tca cta aac      912
```

```
Gly Ala Phe Thr His His Leu Asp Glu Asn Lys Met Ile Ser Leu Asn
    290                 295                 300

ata gat gaa gga ata att ttc aat aaa gtg gta gaa gat ttt gat ttt      960
Ile Asp Glu Gly Ile Ile Phe Asn Lys Val Val Glu Asp Phe Asp Phe
305                 310                 315                 320

aga gca gtg gtt tct tct tta tca gaa tta aaa gga ata gaa tat gaa     1008
Arg Ala Val Val Ser Ser Leu Ser Glu Leu Lys Gly Ile Glu Tyr Glu
                325                 330                 335

gga caa tat att gat aag caa tat gaa gaa ttt att cca tca agt gct     1056
Gly Gln Tyr Ile Asp Lys Gln Tyr Glu Glu Phe Ile Pro Ser Ser Ala
            340                 345                 350

ccc tta tca caa gac cgt cta tgg cag gca gtt gaa agt ttg act caa     1104
Pro Leu Ser Gln Asp Arg Leu Trp Gln Ala Val Glu Ser Leu Thr Gln
        355                 360                 365

agc aat gaa aca atc gtt gct gaa caa gga acc tca ttt ttt gga gct     1152
Ser Asn Glu Thr Ile Val Ala Glu Gln Gly Thr Ser Phe Phe Gly Ala
    370                 375                 380

tca aca att ttc tta aaa tca aat agt cgt ttt att gga caa cct tta     1200
Ser Thr Ile Phe Leu Lys Ser Asn Ser Arg Phe Ile Gly Gln Pro Leu
385                 390                 395                 400

tgg ggt tct att gga tat act ttt cca gcg gct tta gga agc caa att     1248
Trp Gly Ser Ile Gly Tyr Thr Phe Pro Ala Ala Leu Gly Ser Gln Ile
                405                 410                 415

gcg gat aaa gag agc aga cac ctt tta ttt att ggt gat ggt tca ctt     1296
Ala Asp Lys Glu Ser Arg His Leu Leu Phe Ile Gly Asp Gly Ser Leu
            420                 425                 430

caa ctt acc gta caa gaa tta gga cta tca atc aga gaa aaa ctc aat     1344
Gln Leu Thr Val Gln Glu Leu Gly Leu Ser Ile Arg Glu Lys Leu Asn
        435                 440                 445

cca att tgt ttt atc ata aat aat gat ggt tat aca gtt gaa aga gaa     1392
Pro Ile Cys Phe Ile Ile Asn Asn Asp Gly Tyr Thr Val Glu Arg Glu
    450                 455                 460

atc cac gga cct act caa agt tat aac gac att cca atg tgg aat tac     1440
Ile His Gly Pro Thr Gln Ser Tyr Asn Asp Ile Pro Met Trp Asn Tyr
465                 470                 475                 480

tcg aaa tta cca gaa aca ttt gga gca aca gaa gat cgt gta gta tca     1488
Ser Lys Leu Pro Glu Thr Phe Gly Ala Thr Glu Asp Arg Val Val Ser
                485                 490                 495

aaa att gtt aga aca gag aat gaa ttt gtg tct gtc atg aaa gaa gcc     1536
Lys Ile Val Arg Thr Glu Asn Glu Phe Val Ser Val Met Lys Glu Ala
            500                 505                 510

caa gca gat gtc aat aga atg tat tgg ata gaa cta gtt ttg gaa aaa     1584
Gln Ala Asp Val Asn Arg Met Tyr Trp Ile Glu Leu Val Leu Glu Lys
        515                 520                 525
```

gaa gat gcg cca aaa tta ctg aaa aaa atg ggt aaa tta ttt gct gag 1632
Glu Asp Ala Pro Lys Leu Leu Lys Lys Met Gly Lys Leu Phe Ala Glu
530 535 540

caa aat aaa tag 1644
Gln Asn Lys
545

<210> 40
<211> 547
<212> PRT
<213> 乳酸乳球菌

<400> 40

Met Tyr Thr Val Gly Asp Tyr Leu Leu Asp Arg Leu His Glu Leu Gly
1 5 10 15

Ile Glu Glu Ile Phe Gly Val Pro Gly Asp Tyr Asn Leu Gln Phe Leu
20 25 30

Asp Gln Ile Ile Ser Arg Glu Asp Met Lys Trp Ile Gly Asn Ala Asn
35 40 45

Glu Leu Asn Ala Ser Tyr Met Ala Asp Gly Tyr Ala Arg Thr Lys Lys
50 55 60

Ala Ala Ala Phe Leu Thr Thr Phe Gly Val Gly Glu Leu Ser Ala Ile
65 70 75 80

Asn Gly Leu Ala Gly Ser Tyr Ala Glu Asn Leu Pro Val Val Glu Ile
85 90 95

Val Gly Ser Pro Thr Ser Lys Val Gln Asn Asp Gly Lys Phe Val His
100 105 110

His Thr Leu Ala Asp Gly Asp Phe Lys His Phe Met Lys Met His Glu
115 120 125

Pro Val Thr Ala Ala Arg Thr Leu Leu Thr Ala Glu Asn Ala Thr Tyr
130 135 140

Glu Ile Asp Arg Val Leu Ser Gln Leu Leu Lys Glu Arg Lys Pro Val
145 150 155 160

Tyr Ile Asn Leu Pro Val Asp Val Ala Ala Ala Lys Ala Glu Lys Pro
165 170 175

Ala Leu Ser Leu Glu Lys Glu Ser Ser Thr Thr Asn Thr Thr Glu Gln
180 185 190

Val Ile Leu Ser Lys Ile Glu Glu Ser Leu Lys Asn Ala Gln Lys Pro
195 200 205

Val Val Ile Ala Gly His Glu Val Ile Ser Phe Gly Leu Glu Lys Thr
210 215 220

Val Thr Gln Phe Val Ser Glu Thr Lys Leu Pro Ile Thr Thr Leu Asn
225 230 235 240

Phe Gly Lys Ser Ala Val Asp Glu Ser Leu Pro Ser Phe Leu Gly Ile
245 250 255

Tyr Asn Gly Lys Leu Ser Glu Ile Ser Leu Lys Asn Phe Val Glu Ser
260 265 270

Ala Asp Phe Ile Leu Met Leu Gly Val Lys Leu Thr Asp Ser Ser Thr
275 280 285

Gly Ala Phe Thr His His Leu Asp Glu Asn Lys Met Ile Ser Leu Asn
290 295 300

Ile Asp Glu Gly Ile Ile Phe Asn Lys Val Val Glu Asp Phe Asp Phe
305 310 315 320

Arg Ala Val Val Ser Ser Leu Ser Glu Leu Lys Gly Ile Glu Tyr Glu
325 330 335

Gly Gln Tyr Ile Asp Lys Gln Tyr Glu Glu Phe Ile Pro Ser Ser Ala
340 345 350

Pro Leu Ser Gln Asp Arg Leu Trp Gln Ala Val Glu Ser Leu Thr Gln
355 360 365

Ser Asn Glu Thr Ile Val Ala Glu Gln Gly Thr Ser Phe Phe Gly Ala
370 375 380

Ser Thr Ile Phe Leu Lys Ser Asn Ser Arg Phe Ile Gly Gln Pro Leu
385 390 395 400

Trp Gly Ser Ile Gly Tyr Thr Phe Pro Ala Ala Leu Gly Ser Gln Ile
405 410 415

Ala Asp Lys Glu Ser Arg His Leu Leu Phe Ile Gly Asp Gly Ser Leu
420 425 430

Gln Leu Thr Val Gln Glu Leu Gly Leu Ser Ile Arg Glu Lys Leu Asn
435 440 445

Pro Ile Cys Phe Ile Ile Asn Asn Asp Gly Tyr Thr Val Glu Arg Glu
450 455 460

Ile His Gly Pro Thr Gln Ser Tyr Asn Asp Ile Pro Met Trp Asn Tyr
465 470 475 480

Ser Lys Leu Pro Glu Thr Phe Gly Ala Thr Glu Asp Arg Val Val Ser
485 490 495

Lys Ile Val Arg Thr Glu Asn Glu Phe Val Ser Val Met Lys Glu Ala
500 505 510

Gln Ala Asp Val Asn Arg Met Tyr Trp Ile Glu Leu Val Leu Glu Lys
515 520 525

Glu Asp Ala Pro Lys Leu Leu Lys Lys Met Gly Lys Leu Phe Ala Glu
530 535 540

Gln Asn Lys
545

<210> 41
<211> 1644
<212> DNA
<213> 人造

<220>
<223> 乳酸乳球菌分支鏈α酮酸去羧苷 KdcA 密碼子最佳化基因

<400> 41
atgtatactg ttggtgatta tctgctggac cgtctgcatg aactgggcat tgaagaaatc 60

ttcggtgtcc caggcgacta caacctgcag ttcctggacc agatcatctc ccgcgaagat 120

atgaaatgga tcggtaacgc aaacgagctg aacgcgtctt atatggctga tggttatgct 180

cgcaccaaaa aggctgcggc ctttctgacc acctttggtg tgggcgagct gagcgcgatc 240

aacggcctgg caggttccta cgctgagaac ctgccggtag tagaaatcgt tggttccccg 300

acctctaagg ttcagaacga cggcaaattc gtacatcaca ccctggcgga cggcgatttt 360

aagcacttta tgaaaatgca cgaaccggtc accgccgctc gcactctgct gaccgcggaa 420

aacgcaacgt acgagatcga tcgtgtactg tcccagctgc tgaaagaacg taaaccggtg 480

tatatcaatc tgccggttga tgtcgctgcg gccaaagcag agaaaccggc actgtccctg 540

gagaaggaga gctccactac taacaccacc gaacaggtta tcctgtccaa aattgaagaa 600

tctctgaaaa acgcacagaa accggtggtt atcgcaggtc acgaggttat ctccttcggc 660

ctggagaaaa ctgttactca attcgtctct gaaacgaaac tgccgatcac gaccctgaac 720

tttggcaagt ccgcagttga cgaatctctg ccttctttcc tgggcattta caacggcaaa 780

ctgtccgaga tctccctgaa gaacttcgta gaatccgctg actttatcct gatgctgggt 840

gtgaaactga ccgactcctc taccggtgcg ttcacgcacc atctggatga aaacaaaatg 900

atcagcctga acatcgacga gggtatcatc ttcaacaagg tagttgaaga tttcgacttc 960

cgtgctgttg tcagcagcct gtccgagctg aaaggcattg agtacgaggg tcaatacatc 1020

gataaacagt acgaagagtt tattccgtct tctgcaccgc tgagccagga ccgcctgtgg 1080

caggcagttg agtccctgac gcagtccaac gaaactatcg tagcggaaca aggtacctct 1140

ttcttcggtg cttctaccat ctttctgaag tccaactctc gctttatcgg tcagccgctg 1200

tggggttcta tcggttacac gttcccggct gcgctgggta gccagatcgc tgataaagag 1260

tctcgtcatc tgctgttcat cggtgatggt tccctgcagc tgactgtaca ggaactgggt 1320

ctgtctatcc gtgaaaaact gaacccgatt tgttttatca tcaataacga tggctacact 1380

gttgagcgtg aaattcatgg tccgactcag tcttacaacg atattccgat gtggaactac 1440

tctaaactgc cggaaacctt cggtgcaact gaggatcgcg tcgtgagcaa gattgtgcgt 1500

actgagaacg agttcgtatc tgttatgaaa gaggcgcagg cagatgtgaa ccgcatgtac 1560

tggatcgaac tggttctgga aaaagaggat gcaccgaaac tgctgaagaa aatgggtaaa 1620

ctgtttgcgg agcagaacaa gtaa 1644

<210> 42
<211> 1647
<212> DNA
<213> 乳酸乳球菌

<220>
<221> CDS
<222> (1)..(1647)

<400> 42
atg tat aca gta gga gat tac cta tta gac cga tta cac gag tta gga 48
Met Tyr Thr Val Gly Asp Tyr Leu Leu Asp Arg Leu His Glu Leu Gly
1 5 10 15

```
att gaa gaa att ttt gga gtc cct gga gac tat aac tta caa ttt tta      96
Ile Glu Glu Ile Phe Gly Val Pro Gly Asp Tyr Asn Leu Gln Phe Leu
            20                  25                  30

gat caa att att tcc cac aag gat atg aaa tgg gtc gga aat gct aat     144
Asp Gln Ile Ile Ser His Lys Asp Met Lys Trp Val Gly Asn Ala Asn
        35                  40                  45

gaa tta aat gct tca tat atg gct gat ggc tat gct cgt act aaa aaa     192
Glu Leu Asn Ala Ser Tyr Met Ala Asp Gly Tyr Ala Arg Thr Lys Lys
    50                  55                  60

gct gcc gca ttt ctt aca acc ttt gga gta ggt gaa ttg agt gca gtt     240
Ala Ala Ala Phe Leu Thr Thr Phe Gly Val Gly Glu Leu Ser Ala Val
65                  70                  75                  80

aat gga tta gca gga agt tac gcc gaa aat tta cca gta gta gaa ata     288
Asn Gly Leu Ala Gly Ser Tyr Ala Glu Asn Leu Pro Val Val Glu Ile
                85                  90                  95

gtg gga tca cct aca tca aaa gtt caa aat gaa gga aaa ttt gtt cat     336
Val Gly Ser Pro Thr Ser Lys Val Gln Asn Glu Gly Lys Phe Val His
            100                 105                 110

cat acg ctg gct gac ggt gat ttt aaa cac ttt atg aaa atg cac gaa     384
His Thr Leu Ala Asp Gly Asp Phe Lys His Phe Met Lys Met His Glu
        115                 120                 125

cct gtt aca gca gct cga act tta ctg aca gca gaa aat gca acc gtt     432
Pro Val Thr Ala Ala Arg Thr Leu Leu Thr Ala Glu Asn Ala Thr Val
    130                 135                 140

gaa att gac cga gta ctt tct gca cta tta aaa gaa aga aaa cct gtc     480
Glu Ile Asp Arg Val Leu Ser Ala Leu Leu Lys Glu Arg Lys Pro Val
145                 150                 155                 160

tat atc aac tta cca gtt gat gtt gct gct gca aaa gca gag aaa ccc     528
Tyr Ile Asn Leu Pro Val Asp Val Ala Ala Ala Lys Ala Glu Lys Pro
                165                 170                 175

tca ctc cct ttg aaa aag gaa aac tca act tca aat aca agt gac caa     576
Ser Leu Pro Leu Lys Lys Glu Asn Ser Thr Ser Asn Thr Ser Asp Gln
            180                 185                 190

gaa att ttg aac aaa att caa gaa agc ttg aaa aat gcc aaa aaa cca     624
Glu Ile Leu Asn Lys Ile Gln Glu Ser Leu Lys Asn Ala Lys Lys Pro
        195                 200                 205

atc gtg att aca gga cat gaa ata att agt ttt ggc tta gaa aaa aca     672
Ile Val Ile Thr Gly His Glu Ile Ile Ser Phe Gly Leu Glu Lys Thr
    210                 215                 220

gtc act caa ttt att tca aag aca aaa cta cct att acg aca tta aac     720
Val Thr Gln Phe Ile Ser Lys Thr Lys Leu Pro Ile Thr Thr Leu Asn
225                 230                 235                 240

ttt ggt aaa agt tca gtt gat gaa gcc ctc cct tca ttt tta gga atc     768
Phe Gly Lys Ser Ser Val Asp Glu Ala Leu Pro Ser Phe Leu Gly Ile
```

```
                    245                 250                 255

tat aat ggt aca ctc tca gag cct aat ctt aaa gaa ttc gtg gaa tca      816
Tyr Asn Gly Thr Leu Ser Glu Pro Asn Leu Lys Glu Phe Val Glu Ser
            260                 265                 270

gcc gac ttc atc ttg atg ctt gga gtt aaa ctc aca gac tct tca aca      864
Ala Asp Phe Ile Leu Met Leu Gly Val Lys Leu Thr Asp Ser Ser Thr
        275                 280                 285

gga gcc ttc act cat cat tta aat gaa aat aaa atg att tca ctg aat      912
Gly Ala Phe Thr His His Leu Asn Glu Asn Lys Met Ile Ser Leu Asn
    290                 295                 300

ata gat gaa gga aaa ata ttt aac gaa aga atc caa aat ttt gat ttt      960
Ile Asp Glu Gly Lys Ile Phe Asn Glu Arg Ile Gln Asn Phe Asp Phe
305                 310                 315                 320

gaa tcc ctc atc tcc tct ctc tta gac cta agc gaa ata gaa tac aaa     1008
Glu Ser Leu Ile Ser Ser Leu Leu Asp Leu Ser Glu Ile Glu Tyr Lys
                325                 330                 335

gga aaa tat atc gat aaa aag caa gaa gac ttt gtt cca tca aat gcg     1056
Gly Lys Tyr Ile Asp Lys Lys Gln Glu Asp Phe Val Pro Ser Asn Ala
            340                 345                 350

ctt tta tca caa gac cgc cta tgg caa gca gtt gaa aac cta act caa     1104
Leu Leu Ser Gln Asp Arg Leu Trp Gln Ala Val Glu Asn Leu Thr Gln
        355                 360                 365

agc aat gaa aca atc gtt gct gaa caa ggg aca tca ttc ttt ggc gct     1152
Ser Asn Glu Thr Ile Val Ala Glu Gln Gly Thr Ser Phe Phe Gly Ala
    370                 375                 380

tca tca att ttc tta aaa tca aag agt cat ttt att ggt caa ccc tta     1200
Ser Ser Ile Phe Leu Lys Ser Lys Ser His Phe Ile Gly Gln Pro Leu
385                 390                 395                 400

tgg gga tca att gga tat aca ttc cca gca gca tta gga agc caa att     1248
Trp Gly Ser Ile Gly Tyr Thr Phe Pro Ala Ala Leu Gly Ser Gln Ile
                405                 410                 415

gca gat aaa gaa agc aga cac ctt tta ttt att ggt gat ggt tca ctt     1296
Ala Asp Lys Glu Ser Arg His Leu Leu Phe Ile Gly Asp Gly Ser Leu
            420                 425                 430

caa ctt aca gtg caa gaa tta gga tta gca atc aga gaa aaa att aat     1344
Gln Leu Thr Val Gln Glu Leu Gly Leu Ala Ile Arg Glu Lys Ile Asn
        435                 440                 445

cca att tgc ttt att atc aat aat gat ggt tat aca gtc gaa aga gaa     1392
Pro Ile Cys Phe Ile Ile Asn Asn Asp Gly Tyr Thr Val Glu Arg Glu
    450                 455                 460

att cat gga cca aat caa agc tac aat gat att cca atg tgg aat tac     1440
Ile His Gly Pro Asn Gln Ser Tyr Asn Asp Ile Pro Met Trp Asn Tyr
465                 470                 475                 480

tca aaa tta cca gaa tcg ttt gga gca aca gaa gat cga gta gtc tca     1488
```

```
Ser Lys Leu Pro Glu Ser Phe Gly Ala Thr Glu Asp Arg Val Val Ser
                485                 490                 495

aaa atc gtt aga act gaa aat gaa ttt gtg tct gtc atg aaa gaa gct      1536
Lys Ile Val Arg Thr Glu Asn Glu Phe Val Ser Val Met Lys Glu Ala
            500                 505                 510

caa gca gat cca aat aga atg tac tgg att gag tta att ttg gca aaa      1584
Gln Ala Asp Pro Asn Arg Met Tyr Trp Ile Glu Leu Ile Leu Ala Lys
        515                 520                 525

gaa ggt gca cca aaa gta ctg aaa aaa atg ggc aaa cta ttt gct gaa      1632
Glu Gly Ala Pro Lys Val Leu Lys Lys Met Gly Lys Leu Phe Ala Glu
    530                 535                 540

caa aat aaa tca taa                                                 1647
Gln Asn Lys Ser
545
```

<210> 43
<211> 548
<212> PRT
<213> 乳酸乳球菌

<400> 43

```
Met Tyr Thr Val Gly Asp Tyr Leu Leu Asp Arg Leu His Glu Leu Gly
1               5                   10                  15

Ile Glu Glu Ile Phe Gly Val Pro Gly Asp Tyr Asn Leu Gln Phe Leu
            20                  25                  30

Asp Gln Ile Ile Ser His Lys Asp Met Lys Trp Val Gly Asn Ala Asn
        35                  40                  45

Glu Leu Asn Ala Ser Tyr Met Ala Asp Gly Tyr Ala Arg Thr Lys Lys
    50                  55                  60

Ala Ala Ala Phe Leu Thr Thr Phe Gly Val Gly Glu Leu Ser Ala Val
65                  70                  75                  80

Asn Gly Leu Ala Gly Ser Tyr Ala Glu Asn Leu Pro Val Val Glu Ile
                85                  90                  95

Val Gly Ser Pro Thr Ser Lys Val Gln Asn Glu Gly Lys Phe Val His
            100                 105                 110

His Thr Leu Ala Asp Gly Asp Phe Lys His Phe Met Lys Met His Glu
        115                 120                 125
```

Pro Val Thr Ala Ala Arg Thr Leu Leu Thr Ala Glu Asn Ala Thr Val
130 135 140

Glu Ile Asp Arg Val Leu Ser Ala Leu Leu Lys Glu Arg Lys Pro Val
145 150 155 160

Tyr Ile Asn Leu Pro Val Asp Val Ala Ala Ala Lys Ala Glu Lys Pro
165 170 175

Ser Leu Pro Leu Lys Lys Glu Asn Ser Thr Ser Asn Thr Ser Asp Gln
180 185 190

Glu Ile Leu Asn Lys Ile Gln Glu Ser Leu Lys Asn Ala Lys Lys Pro
195 200 205

Ile Val Ile Thr Gly His Glu Ile Ile Ser Phe Gly Leu Glu Lys Thr
210 215 220

Val Thr Gln Phe Ile Ser Lys Thr Lys Leu Pro Ile Thr Thr Leu Asn
225 230 235 240

Phe Gly Lys Ser Ser Val Asp Glu Ala Leu Pro Ser Phe Leu Gly Ile
245 250 255

Tyr Asn Gly Thr Leu Ser Glu Pro Asn Leu Lys Glu Phe Val Glu Ser
260 265 270

Ala Asp Phe Ile Leu Met Leu Gly Val Lys Leu Thr Asp Ser Ser Thr
275 280 285

Gly Ala Phe Thr His His Leu Asn Glu Asn Lys Met Ile Ser Leu Asn
290 295 300

Ile Asp Glu Gly Lys Ile Phe Asn Glu Arg Ile Gln Asn Phe Asp Phe
305 310 315 320

Glu Ser Leu Ile Ser Ser Leu Leu Asp Leu Ser Glu Ile Glu Tyr Lys
325 330 335

Gly Lys Tyr Ile Asp Lys Lys Gln Glu Asp Phe Val Pro Ser Asn Ala
340 345 350

Leu Leu Ser Gln Asp Arg Leu Trp Gln Ala Val Glu Asn Leu Thr Gln
355 360 365

Ser Asn Glu Thr Ile Val Ala Glu Gln Gly Thr Ser Phe Phe Gly Ala
370 375 380

Ser Ser Ile Phe Leu Lys Ser Lys Ser His Phe Ile Gly Gln Pro Leu
385 390 395 400

Trp Gly Ser Ile Gly Tyr Thr Phe Pro Ala Ala Leu Gly Ser Gln Ile
405 410 415

Ala Asp Lys Glu Ser Arg His Leu Leu Phe Ile Gly Asp Gly Ser Leu
420 425 430

Gln Leu Thr Val Gln Glu Leu Gly Leu Ala Ile Arg Glu Lys Ile Asn
435 440 445

Pro Ile Cys Phe Ile Ile Asn Asn Asp Gly Tyr Thr Val Glu Arg Glu
450 455 460

Ile His Gly Pro Asn Gln Ser Tyr Asn Asp Ile Pro Met Trp Asn Tyr
465 470 475 480

Ser Lys Leu Pro Glu Ser Phe Gly Ala Thr Glu Asp Arg Val Val Ser
485 490 495

Lys Ile Val Arg Thr Glu Asn Glu Phe Val Ser Val Met Lys Glu Ala
500 505 510

Gln Ala Asp Pro Asn Arg Met Tyr Trp Ile Glu Leu Ile Leu Ala Lys
515 520 525

Glu Gly Ala Pro Lys Val Leu Lys Lys Met Gly Lys Leu Phe Ala Glu
530 535 540

Gln Asn Lys Ser
545

<210> 44
<211> 1647
<212> DNA
<213> 人造

<220>
<223> 乳酸乳球菌α酮異戊酸去羧苷 KivD 密碼子最佳化基因

<400> 44
atgtatactg ttggtgatta cctgctggat cgtctgcatg aactgggcat cgaggaaatt 60

ttcggcgtac ctggtgacta taacctgcag ttcctggatc agatcatttc ccacaaagat 120
atgaaatggg ttggtaacgc gaacgagctg aatgcaagct acatggctga cggttatgca 180
cgcaccaaga aagctgcggc gttcctgact acttttggcg tcggcgagct gtctgcggta 240
aacggtctgg ccggctccta cgcggaaaac ctgccggtag tagaaatcgt cggttccccg 300
acctctaaag ttcagaacga gggtaaattc gtgcaccata ctctggccga tggtgacttc 360
aaacacttca tgaagatgca cgaaccggtc actgctgctc gtacgctgct gaccgcggaa 420
aatgcgactg tcgagattga tcgtgtactg agcgcactgc tgaaagaacg caagcctgta 480
tacatcaacc tgccggttga tgtcgcggcc gccaaagcgg aaaaaccatc tctgccgctg 540
aaaaaggaga acagcacctc taacaccagc gaccaggaaa tcctgaacaa gatccaggag 600
tctctgaaga acgctaaaaa gccgatcgta atcaccggcc atgagattat ctctttcggt 660
ctggagaaaa ctgtcaccca gttcatcagc aaaaccaaac tgccgatcac caccctgaac 720
ttcggtaaat cctccgttga cgaagcgctg ccgtcctttc tgggtattta caacggcact 780
ctgtctgagc cgaacctgaa agagttcgtg gagtctgcgg attttatcct gatgctgggc 840
gtgaaactga cggattcctc caccggtgca ttcacccacc acctgaatga gaataaaatg 900
atctctctga acattgatga gggcaaaatc ttcaacgagc gtattcagaa cttcgatttc 960
gaatccctga tctcctccct gctggatctg tccgagattg aatataaagg caaatacatt 1020
gataagaagc aagaggactt cgtaccgtct aacgcgctgc tgagccagga ccgtctgtgg 1080
caagctgtgg aaaacctgac ccagtccaac gaaaccatcg tggcggaaca gggtacctcc 1140
ttcttcggtg ctagctctat cttcctgaaa tctaaaagcc acttcatcgg tcagccactg 1200
tggggctcta ttggctacac cttcccggca gcgctgggtt cccaaatcgc agacaaagaa 1260
tcccgccacc tgctgttcat tggtgacggc tctctgcaac tgaccgtaca ggagctgggt 1320
ctggcgattc gtgagaaaat caacccgatt tgtttcatca tcaacaacga tggctacact 1380
gttgagcgtg agatccacgg cccgaaccag tcctacaacg acattccgat gtggaactac 1440
tctaaactgc cggaatcctt cggtgcgact gaagaccgtg tcgtaagcaa gatcgtccgt 1500
accgaaaacg aattcgtgtc tgtcatgaaa gaagcacagg cggacccgaa ccgcatgtac 1560
tggatcgagc tgattctggc taaagagggc gcgccaaaag tactgaaaaa gatgggtaaa 1620
ctgttcgcag aacagaacaa atcctaa 1647

<210> 45
<211> 3696
<212> DNA

<213> 結核分枝桿菌

<220>
<221> CDS
<222> (1)..(3696)

<400> 45

```
gtg gcc aac ata agt tca cca ttc ggg caa aac gaa tgg ctg gtc gaa      48
Val Ala Asn Ile Ser Ser Pro Phe Gly Gln Asn Glu Trp Leu Val Glu
1               5                   10                  15

gag atg tac cgc aag ttc cgc gac gac ccc tcc tcg gtc gat ccc agc      96
Glu Met Tyr Arg Lys Phe Arg Asp Asp Pro Ser Ser Val Asp Pro Ser
            20                  25                  30

tgg cac gag ttc ctg gtt gac tac agc ccc gaa ccc acc tcc caa cca     144
Trp His Glu Phe Leu Val Asp Tyr Ser Pro Glu Pro Thr Ser Gln Pro
        35                  40                  45

gct gcc gaa cca acc cgg gtt acc tcg cca ctc gtt gcc gag cgg gcc     192
Ala Ala Glu Pro Thr Arg Val Thr Ser Pro Leu Val Ala Glu Arg Ala
    50                  55                  60

gct gcg gcc gcc ccg cag gca ccc ccc aag ccg gcc gac acc gcg gcc     240
Ala Ala Ala Ala Pro Gln Ala Pro Pro Lys Pro Ala Asp Thr Ala Ala
65                  70                  75                  80

gcg ggc aac ggc gtg gtc gcc gca ctg gcc gcc aaa act gcc gtt ccc     288
Ala Gly Asn Gly Val Val Ala Ala Leu Ala Ala Lys Thr Ala Val Pro
                85                  90                  95

ccg cca gcc gaa ggt gac gag gta gcg gtg ctg cgc ggc gcc gcc gcg     336
Pro Pro Ala Glu Gly Asp Glu Val Ala Val Leu Arg Gly Ala Ala Ala
            100                 105                 110

gcc gtc gtc aag aac atg tcc gcg tcg ttg gag gtg ccg acg gcg acc     384
Ala Val Val Lys Asn Met Ser Ala Ser Leu Glu Val Pro Thr Ala Thr
        115                 120                 125

agc gtc cgg gcg gtc ccg gcc aag cta ctg atc gac aac cgg atc gtc     432
Ser Val Arg Ala Val Pro Ala Lys Leu Leu Ile Asp Asn Arg Ile Val
    130                 135                 140

atc aac aac cag ttg aag cgg acc cgc ggc ggc aag atc tcg ttc acg     480
Ile Asn Asn Gln Leu Lys Arg Thr Arg Gly Gly Lys Ile Ser Phe Thr
145                 150                 155                 160

cat ttg ctg ggc tac gcc ctg gtg cag gcg gtg aag aaa ttc ccg aac     528
His Leu Leu Gly Tyr Ala Leu Val Gln Ala Val Lys Lys Phe Pro Asn
                165                 170                 175

atg aac cgg cac tac acc gaa gtc gac ggc aag ccc acc gcg gtc acg     576
Met Asn Arg His Tyr Thr Glu Val Asp Gly Lys Pro Thr Ala Val Thr
            180                 185                 190

ccg gcg cac acc aat ctc ggc ctg gcg atc gac ctg caa ggc aag gac     624
Pro Ala His Thr Asn Leu Gly Leu Ala Ile Asp Leu Gln Gly Lys Asp
        195                 200                 205
```

```
ggg aag cgt tcc ctg gtg gtg gcc ggc atc aag cgg tgc gag acc atg      672
Gly Lys Arg Ser Leu Val Val Ala Gly Ile Lys Arg Cys Glu Thr Met
    210                 215                 220

cga ttc gcg cag ttc gtc acg gcc tac gaa gac atc gta cgc cgg gcc      720
Arg Phe Ala Gln Phe Val Thr Ala Tyr Glu Asp Ile Val Arg Arg Ala
225                 230                 235                 240

cgc gac ggc aag ctg acc act gaa gac ttt gcc ggc gtg acg att tcg      768
Arg Asp Gly Lys Leu Thr Thr Glu Asp Phe Ala Gly Val Thr Ile Ser
                245                 250                 255

ctg acc aat ccc gga acc atc ggc acc gtg cat tcg gtg ccg cgg ctg      816
Leu Thr Asn Pro Gly Thr Ile Gly Thr Val His Ser Val Pro Arg Leu
            260                 265                 270

atg ccc ggc cag ggc gcc atc atc ggc gtg ggc gcc atg gaa tac ccc      864
Met Pro Gly Gln Gly Ala Ile Ile Gly Val Gly Ala Met Glu Tyr Pro
        275                 280                 285

gcc gag ttt caa ggc gcc agc gag gaa cgc atc gcc gag ctg ggc atc      912
Ala Glu Phe Gln Gly Ala Ser Glu Glu Arg Ile Ala Glu Leu Gly Ile
    290                 295                 300

ggc aaa ttg atc act ttg acc tcc acc tac gac cac cgc atc atc cag      960
Gly Lys Leu Ile Thr Leu Thr Ser Thr Tyr Asp His Arg Ile Ile Gln
305                 310                 315                 320

ggc gcg gaa tcg ggc gac ttc ctg cgc acc atc cac gag ttg ctg ctc     1008
Gly Ala Glu Ser Gly Asp Phe Leu Arg Thr Ile His Glu Leu Leu Leu
                325                 330                 335

tcg gat ggc ttc tgg gac gag gtc ttc cgc gaa ctg agc atc cca tat     1056
Ser Asp Gly Phe Trp Asp Glu Val Phe Arg Glu Leu Ser Ile Pro Tyr
            340                 345                 350

ctg ccg gtg cgc tgg agc acc gac aac ccc gac tcg atc gtc gac aag     1104
Leu Pro Val Arg Trp Ser Thr Asp Asn Pro Asp Ser Ile Val Asp Lys
        355                 360                 365

aac gct cgc gtc atg aac ttg atc gcg gcc tac cgc aac cgc ggc cat     1152
Asn Ala Arg Val Met Asn Leu Ile Ala Ala Tyr Arg Asn Arg Gly His
    370                 375                 380

ctg atg gcc gat acc gac ccg ctg cgg ttg gac aaa gct cgg ttc cgc     1200
Leu Met Ala Asp Thr Asp Pro Leu Arg Leu Asp Lys Ala Arg Phe Arg
385                 390                 395                 400

agt cac ccc gac ctc gaa gtg ctg acc cac ggc ctg acg ctg tgg gat     1248
Ser His Pro Asp Leu Glu Val Leu Thr His Gly Leu Thr Leu Trp Asp
                405                 410                 415

ctc gat cgg gtg ttc aag gtc gac ggc ttt gcc ggt gcg cag tac aag     1296
Leu Asp Arg Val Phe Lys Val Asp Gly Phe Ala Gly Ala Gln Tyr Lys
            420                 425                 430

aaa ctg cgc gac gtg ctg ggc ttg ctg cgc gat gcc tac tgc cgc cac     1344
Lys Leu Arg Asp Val Leu Gly Leu Leu Arg Asp Ala Tyr Cys Arg His
```

```
          435                 440                 445

atc ggc gtg gag tac gcc cat atc ctc gac ccc gaa caa aag gag tgg   1392
Ile Gly Val Glu Tyr Ala His Ile Leu Asp Pro Glu Gln Lys Glu Trp
    450                 455                 460

ctc gaa caa cgg gtc gag acc aag cac gtc aaa ccc act gtg gcc caa   1440
Leu Glu Gln Arg Val Glu Thr Lys His Val Lys Pro Thr Val Ala Gln
465                 470                 475                 480

cag aaa tac atc ctc agc aag ctc aac gcc gcc gag gcc ttt gaa acg   1488
Gln Lys Tyr Ile Leu Ser Lys Leu Asn Ala Ala Glu Ala Phe Glu Thr
                485                 490                 495

ttc cta cag acc aag tac gtc ggc cag aag cgg ttc tcg ctg gaa ggc   1536
Phe Leu Gln Thr Lys Tyr Val Gly Gln Lys Arg Phe Ser Leu Glu Gly
            500                 505                 510

gcc gaa agc gtg atc ccg atg atg gac gcg gcg atc gac cag tgc gct   1584
Ala Glu Ser Val Ile Pro Met Met Asp Ala Ala Ile Asp Gln Cys Ala
        515                 520                 525

gag cac ggc ctc gac gag gtg gtc atc ggg atg ccg cac cgg ggc cgg   1632
Glu His Gly Leu Asp Glu Val Val Ile Gly Met Pro His Arg Gly Arg
    530                 535                 540

ctc aac gtg ctg gcc aac atc gtc ggc aag ccg tac tcg cag atc ttc   1680
Leu Asn Val Leu Ala Asn Ile Val Gly Lys Pro Tyr Ser Gln Ile Phe
545                 550                 555                 560

acc gag ttc gag ggc aac ctg aat ccg tcg cag gcg cac ggc tcc ggt   1728
Thr Glu Phe Glu Gly Asn Leu Asn Pro Ser Gln Ala His Gly Ser Gly
                565                 570                 575

gac gtc aag tac cac ctg ggc gcc acc ggg ctg tac ctg cag atg ttc   1776
Asp Val Lys Tyr His Leu Gly Ala Thr Gly Leu Tyr Leu Gln Met Phe
            580                 585                 590

ggc gac aac gac att cag gtg tcg ctg acc gcc aac ccg tcg cat ctg   1824
Gly Asp Asn Asp Ile Gln Val Ser Leu Thr Ala Asn Pro Ser His Leu
        595                 600                 605

gag gcc gtc gac ccg gtg ctg gag gga ttg gtg cgg gcc aag cag gat   1872
Glu Ala Val Asp Pro Val Leu Glu Gly Leu Val Arg Ala Lys Gln Asp
    610                 615                 620

ctg ctc gac cac gga agc atc gac agc gac ggc caa cgg gcg ttc tcg   1920
Leu Leu Asp His Gly Ser Ile Asp Ser Asp Gly Gln Arg Ala Phe Ser
625                 630                 635                 640

gtg gtg ccg ctg atg ttg cat ggc gat gcc gcg ttc gcc ggt cag ggt   1968
Val Val Pro Leu Met Leu His Gly Asp Ala Ala Phe Ala Gly Gln Gly
                645                 650                 655

gtg gtc gcc gag acg ctg aac ctg gcg aat ctg ccg ggc tac cgc gtc   2016
Val Val Ala Glu Thr Leu Asn Leu Ala Asn Leu Pro Gly Tyr Arg Val
            660                 665                 670

ggc ggc acc atc cac atc atc gtc aac aac cag atc ggc ttc acc acc   2064
```

```
Gly Gly Thr Ile His Ile Ile Val Asn Asn Gln Ile Gly Phe Thr Thr
        675                 680                 685

gcg ccc gag tat tcc agg tcc agc gag tac tgc acc gac gtc gca aag      2112
Ala Pro Glu Tyr Ser Arg Ser Ser Glu Tyr Cys Thr Asp Val Ala Lys
    690                 695                 700

atg atc ggg gca ccg atc ttt cac gtc aac ggc gac gac ccg gag gcg      2160
Met Ile Gly Ala Pro Ile Phe His Val Asn Gly Asp Asp Pro Glu Ala
705                 710                 715                 720

tgt gtc tgg gtg gcg cgg ttg gcg gtg gac ttc cga caa cgg ttc aag      2208
Cys Val Trp Val Ala Arg Leu Ala Val Asp Phe Arg Gln Arg Phe Lys
                725                 730                 735

aag gac gtc gtc atc gac atg ctg tgc tac cgc cgc cgc ggg cac aac      2256
Lys Asp Val Val Ile Asp Met Leu Cys Tyr Arg Arg Arg Gly His Asn
            740                 745                 750

gag ggt gac gac ccg tcg atg acc aac ccc tac gtg tac gac gtc gtc      2304
Glu Gly Asp Asp Pro Ser Met Thr Asn Pro Tyr Val Tyr Asp Val Val
        755                 760                 765

gac acc aag cgc ggg gcc cgc aaa agc tac acc gaa gcc ctg atc gga      2352
Asp Thr Lys Arg Gly Ala Arg Lys Ser Tyr Thr Glu Ala Leu Ile Gly
    770                 775                 780

cgt ggc gac atc tcg atg aag gag gcc gag gac gcg ctg cgc gac tac      2400
Arg Gly Asp Ile Ser Met Lys Glu Ala Glu Asp Ala Leu Arg Asp Tyr
785                 790                 795                 800

cag ggc cag ctg gaa cgg gtg ttc aac gaa gtg cgc gag ctg gag aag      2448
Gln Gly Gln Leu Glu Arg Val Phe Asn Glu Val Arg Glu Leu Glu Lys
                805                 810                 815

cac ggt gtg cag ccg agc gag tcg gtc gag tcc gac cag atg att ccc      2496
His Gly Val Gln Pro Ser Glu Ser Val Glu Ser Asp Gln Met Ile Pro
            820                 825                 830

gcg ggg ctg gcc act gcg gtg gac aag tcg ctg ctg gcc cgg atc ggc      2544
Ala Gly Leu Ala Thr Ala Val Asp Lys Ser Leu Leu Ala Arg Ile Gly
        835                 840                 845

gat gcg ttc ctc gcc ttg ccg aac ggc ttc acc gcg cac ccg cga gtc      2592
Asp Ala Phe Leu Ala Leu Pro Asn Gly Phe Thr Ala His Pro Arg Val
    850                 855                 860

caa ccg gtg ctg gag aag cgc cgg gag atg gcc tat gaa ggc aag atc      2640
Gln Pro Val Leu Glu Lys Arg Arg Glu Met Ala Tyr Glu Gly Lys Ile
865                 870                 875                 880

gac tgg gcc ttt ggc gag ctg ctg gcg ctg ggc tcg ctg gtg gcc gaa      2688
Asp Trp Ala Phe Gly Glu Leu Leu Ala Leu Gly Ser Leu Val Ala Glu
                885                 890                 895

ggc aag ctg gtg cgc ttg tcg ggg cag gac agc cgc cgc ggc acc ttc      2736
Gly Lys Leu Val Arg Leu Ser Gly Gln Asp Ser Arg Arg Gly Thr Phe
            900                 905                 910
```

```
tcc cag cgg cat tcg gtt ctc atc gac cgc cac act ggc gag gag ttc      2784
Ser Gln Arg His Ser Val Leu Ile Asp Arg His Thr Gly Glu Glu Phe
        915                 920                 925

aca cca ctg cag ctg ctg gcg acc aac tcc gac ggc agc ccg acc ggc      2832
Thr Pro Leu Gln Leu Leu Ala Thr Asn Ser Asp Gly Ser Pro Thr Gly
    930                 935                 940

gga aag ttc ctg gtc tac gac tcg cca ctg tcg gag tac gcc gcc gtc      2880
Gly Lys Phe Leu Val Tyr Asp Ser Pro Leu Ser Glu Tyr Ala Ala Val
945                 950                 955                 960

ggc ttc gag tac ggc tac act gtg ggc aat ccg gac gcc gtg gtg ctc      2928
Gly Phe Glu Tyr Gly Tyr Thr Val Gly Asn Pro Asp Ala Val Val Leu
                965                 970                 975

tgg gag gcg cag ttc ggc gac ttc gtc aac ggc gcg cag tcg atc atc      2976
Trp Glu Ala Gln Phe Gly Asp Phe Val Asn Gly Ala Gln Ser Ile Ile
            980                 985                 990

gac gag ttc atc agc tcc ggt gag  gcc aag tgg ggc caa  ttg tcc aac   3024
Asp Glu Phe Ile Ser Ser Gly Glu  Ala Lys Trp Gly Gln  Leu Ser Asn
        995                 1000                1005

gtc gtg  ctg ctg tta ccg cac  ggg cac gag ggg cag  gga ccc gac      3069
Val Val  Leu Leu Leu Pro His  Gly His Glu Gly Gln  Gly Pro Asp
    1010                1015                1020

cac act  tct gcc cgg atc gaa  cgc ttc ttg cag ttg  tgg gcg gaa      3114
His Thr  Ser Ala Arg Ile Glu  Arg Phe Leu Gln Leu  Trp Ala Glu
    1025                1030                1035

ggt tcg  atg acc atc gcg atg  ccg tcg act ccg tcg  aac tac ttc      3159
Gly Ser  Met Thr Ile Ala Met  Pro Ser Thr Pro Ser  Asn Tyr Phe
    1040                1045                1050

cac ctg  cta cgc cgg cat gcc  ctg gac ggc atc caa  cgc ccg ctg      3204
His Leu  Leu Arg Arg His Ala  Leu Asp Gly Ile Gln  Arg Pro Leu
    1055                1060                1065

atc gtg  ttc acg ccc aag tcg  atg ttg cgt cac aag  gcc gcc gtc      3249
Ile Val  Phe Thr Pro Lys Ser  Met Leu Arg His Lys  Ala Ala Val
    1070                1075                1080

agc gaa  atc aag gac ttc acc  gag atc aag ttc cgc  tca gtg ctg      3294
Ser Glu  Ile Lys Asp Phe Thr  Glu Ile Lys Phe Arg  Ser Val Leu
    1085                1090                1095

gag gaa  ccc acc tat gag gac  ggc atc gga gac cgc  aac aag gtc      3339
Glu Glu  Pro Thr Tyr Glu Asp  Gly Ile Gly Asp Arg  Asn Lys Val
    1100                1105                1110

agc cgg  atc ctg ctg acc agt  ggc aag ctg tat tac  gag ctg gcc      3384
Ser Arg  Ile Leu Leu Thr Ser  Gly Lys Leu Tyr Tyr  Glu Leu Ala
    1115                1120                1125

gcc cgc  aag gcc aag gac aac  cgc aat gac ctc gcg  atc gtg cgg      3429
Ala Arg  Lys Ala Lys Asp Asn  Arg Asn Asp Leu Ala  Ile Val Arg
    1130                1135                1140
```

```
ctt gaa  cag ctc gcc ccg ctg  ccc agg cgt cga ctg  cgt gaa acg      3474
Leu Glu  Gln Leu Ala Pro Leu  Pro Arg Arg Arg Leu  Arg Glu Thr
    1145                 1150                 1155

ctg gac  cgc tac gag aac gtc  aag gag ttc ttc tgg  gtc caa gag      3519
Leu Asp  Arg Tyr Glu Asn Val  Lys Glu Phe Phe Trp  Val Gln Glu
    1160                 1165                 1170

gaa ccg  gcc aac cag ggt gcg  tgg ccg cga ttc ggg  ctc gaa cta      3564
Glu Pro  Ala Asn Gln Gly Ala  Trp Pro Arg Phe Gly  Leu Glu Leu
    1175                 1180                 1185

ccc gag  ctg ctg cct gac aag  ttg gcc ggg atc aag  cga atc tcg      3609
Pro Glu  Leu Leu Pro Asp Lys  Leu Ala Gly Ile Lys  Arg Ile Ser
    1190                 1195                 1200

cgc cgg  gcg atg tca gcc ccg  tcg tca ggc tcg tcg  aag gtg cac      3654
Arg Arg  Ala Met Ser Ala Pro  Ser Ser Gly Ser Ser  Lys Val His
    1205                 1210                 1215

gcc gtc  gaa cag cag gag atc  ctc gac gag gcg ttc  ggc tga         3696
Ala Val  Glu Gln Gln Glu Ile  Leu Asp Glu Ala Phe  Gly
    1220                 1225                 1230
```

<210> 46
<211> 1231
<212> PRT
<213> 結核分枝桿菌

<400> 46

```
Val Ala Asn Ile Ser Ser Pro Phe Gly Gln Asn Glu Trp Leu Val Glu
1               5                  10                  15

Glu Met Tyr Arg Lys Phe Arg Asp Asp Pro Ser Ser Val Asp Pro Ser
            20                  25                  30

Trp His Glu Phe Leu Val Asp Tyr Ser Pro Glu Pro Thr Ser Gln Pro
        35                  40                  45

Ala Ala Glu Pro Thr Arg Val Thr Ser Pro Leu Val Ala Glu Arg Ala
    50                  55                  60

Ala Ala Ala Ala Pro Gln Ala Pro Pro Lys Pro Ala Asp Thr Ala Ala
65                  70                  75                  80

Ala Gly Asn Gly Val Val Ala Ala Leu Ala Ala Lys Thr Ala Val Pro
                85                  90                  95

Pro Pro Ala Glu Gly Asp Glu Val Ala Val Leu Arg Gly Ala Ala Ala
            100                 105                 110
```

Ala Val Val Lys Asn Met Ser Ala Ser Leu Glu Val Pro Thr Ala Thr
115 120 125

Ser Val Arg Ala Val Pro Ala Lys Leu Leu Ile Asp Asn Arg Ile Val
130 135 140

Ile Asn Asn Gln Leu Lys Arg Thr Arg Gly Gly Lys Ile Ser Phe Thr
145 150 155 160

His Leu Leu Gly Tyr Ala Leu Val Gln Ala Val Lys Lys Phe Pro Asn
165 170 175

Met Asn Arg His Tyr Thr Glu Val Asp Gly Lys Pro Thr Ala Val Thr
180 185 190

Pro Ala His Thr Asn Leu Gly Leu Ala Ile Asp Leu Gln Gly Lys Asp
195 200 205

Gly Lys Arg Ser Leu Val Val Ala Gly Ile Lys Arg Cys Glu Thr Met
210 215 220

Arg Phe Ala Gln Phe Val Thr Ala Tyr Glu Asp Ile Val Arg Arg Ala
225 230 235 240

Arg Asp Gly Lys Leu Thr Thr Glu Asp Phe Ala Gly Val Thr Ile Ser
245 250 255

Leu Thr Asn Pro Gly Thr Ile Gly Thr Val His Ser Val Pro Arg Leu
260 265 270

Met Pro Gly Gln Gly Ala Ile Ile Gly Val Gly Ala Met Glu Tyr Pro
275 280 285

Ala Glu Phe Gln Gly Ala Ser Glu Glu Arg Ile Ala Glu Leu Gly Ile
290 295 300

Gly Lys Leu Ile Thr Leu Thr Ser Thr Tyr Asp His Arg Ile Ile Gln
305 310 315 320

Gly Ala Glu Ser Gly Asp Phe Leu Arg Thr Ile His Glu Leu Leu Leu
325 330 335

Ser Asp Gly Phe Trp Asp Glu Val Phe Arg Glu Leu Ser Ile Pro Tyr

340 345 350

Leu Pro Val Arg Trp Ser Thr Asp Asn Pro Asp Ser Ile Val Asp Lys
355 360 365

Asn Ala Arg Val Met Asn Leu Ile Ala Ala Tyr Arg Asn Arg Gly His
370 375 380

Leu Met Ala Asp Thr Asp Pro Leu Arg Leu Asp Lys Ala Arg Phe Arg
385 390 395 400

Ser His Pro Asp Leu Glu Val Leu Thr His Gly Leu Thr Leu Trp Asp
405 410 415

Leu Asp Arg Val Phe Lys Val Asp Gly Phe Ala Gly Ala Gln Tyr Lys
420 425 430

Lys Leu Arg Asp Val Leu Gly Leu Leu Arg Asp Ala Tyr Cys Arg His
435 440 445

Ile Gly Val Glu Tyr Ala His Ile Leu Asp Pro Glu Gln Lys Glu Trp
450 455 460

Leu Glu Gln Arg Val Glu Thr Lys His Val Lys Pro Thr Val Ala Gln
465 470 475 480

Gln Lys Tyr Ile Leu Ser Lys Leu Asn Ala Ala Glu Ala Phe Glu Thr
485 490 495

Phe Leu Gln Thr Lys Tyr Val Gly Gln Lys Arg Phe Ser Leu Glu Gly
500 505 510

Ala Glu Ser Val Ile Pro Met Met Asp Ala Ala Ile Asp Gln Cys Ala
515 520 525

Glu His Gly Leu Asp Glu Val Val Ile Gly Met Pro His Arg Gly Arg
530 535 540

Leu Asn Val Leu Ala Asn Ile Val Gly Lys Pro Tyr Ser Gln Ile Phe
545 550 555 560

Thr Glu Phe Glu Gly Asn Leu Asn Pro Ser Gln Ala His Gly Ser Gly
565 570 575

Asp Val Lys Tyr His Leu Gly Ala Thr Gly Leu Tyr Leu Gln Met Phe
580 585 590

Gly Asp Asn Asp Ile Gln Val Ser Leu Thr Ala Asn Pro Ser His Leu
595 600 605

Glu Ala Val Asp Pro Val Leu Glu Gly Leu Val Arg Ala Lys Gln Asp
610 615 620

Leu Leu Asp His Gly Ser Ile Asp Ser Asp Gly Gln Arg Ala Phe Ser
625 630 635 640

Val Val Pro Leu Met Leu His Gly Asp Ala Ala Phe Ala Gly Gln Gly
645 650 655

Val Val Ala Glu Thr Leu Asn Leu Ala Asn Leu Pro Gly Tyr Arg Val
660 665 670

Gly Gly Thr Ile His Ile Ile Val Asn Asn Gln Ile Gly Phe Thr Thr
675 680 685

Ala Pro Glu Tyr Ser Arg Ser Ser Glu Tyr Cys Thr Asp Val Ala Lys
690 695 700

Met Ile Gly Ala Pro Ile Phe His Val Asn Gly Asp Asp Pro Glu Ala
705 710 715 720

Cys Val Trp Val Ala Arg Leu Ala Val Asp Phe Arg Gln Arg Phe Lys
725 730 735

Lys Asp Val Val Ile Asp Met Leu Cys Tyr Arg Arg Arg Gly His Asn
740 745 750

Glu Gly Asp Asp Pro Ser Met Thr Asn Pro Tyr Val Tyr Asp Val Val
755 760 765

Asp Thr Lys Arg Gly Ala Arg Lys Ser Tyr Thr Glu Ala Leu Ile Gly
770 775 780

Arg Gly Asp Ile Ser Met Lys Glu Ala Glu Asp Ala Leu Arg Asp Tyr
785 790 795 800

Gln Gly Gln Leu Glu Arg Val Phe Asn Glu Val Arg Glu Leu Glu Lys
805 810 815

```
His Gly Val Gln Pro Ser Glu Ser Val Glu Ser Asp Gln Met Ile Pro
            820                 825                 830

Ala Gly Leu Ala Thr Ala Val Asp Lys Ser Leu Leu Ala Arg Ile Gly
        835                 840                 845

Asp Ala Phe Leu Ala Leu Pro Asn Gly Phe Thr Ala His Pro Arg Val
    850                 855                 860

Gln Pro Val Leu Glu Lys Arg Arg Glu Met Ala Tyr Glu Gly Lys Ile
865                 870                 875                 880

Asp Trp Ala Phe Gly Glu Leu Leu Ala Leu Gly Ser Leu Val Ala Glu
                885                 890                 895

Gly Lys Leu Val Arg Leu Ser Gly Gln Asp Ser Arg Arg Gly Thr Phe
            900                 905                 910

Ser Gln Arg His Ser Val Leu Ile Asp Arg His Thr Gly Glu Glu Phe
        915                 920                 925

Thr Pro Leu Gln Leu Leu Ala Thr Asn Ser Asp Gly Ser Pro Thr Gly
    930                 935                 940

Gly Lys Phe Leu Val Tyr Asp Ser Pro Leu Ser Glu Tyr Ala Ala Val
945                 950                 955                 960

Gly Phe Glu Tyr Gly Tyr Thr Val Gly Asn Pro Asp Ala Val Val Leu
                965                 970                 975

Trp Glu Ala Gln Phe Gly Asp Phe Val Asn Gly Ala Gln Ser Ile Ile
            980                 985                 990

Asp Glu Phe Ile Ser Ser Gly Glu  Ala Lys Trp Gly Gln  Leu Ser Asn
        995                 1000                1005

Val Val  Leu Leu Leu Pro His  Gly His Glu Gly Gln  Gly Pro Asp
    1010                1015                1020

His Thr  Ser Ala Arg Ile Glu  Arg Phe Leu Gln Leu  Trp Ala Glu
    1025                1030                1035

Gly Ser  Met Thr Ile Ala Met  Pro Ser Thr Pro Ser  Asn Tyr Phe
    1040                1045                1050
```

His Leu Leu Arg Arg His Ala Leu Asp Gly Ile Gln Arg Pro Leu
1055 1060 1065

Ile Val Phe Thr Pro Lys Ser Met Leu Arg His Lys Ala Ala Val
1070 1075 1080

Ser Glu Ile Lys Asp Phe Thr Glu Ile Lys Phe Arg Ser Val Leu
1085 1090 1095

Glu Glu Pro Thr Tyr Glu Asp Gly Ile Gly Asp Arg Asn Lys Val
1100 1105 1110

Ser Arg Ile Leu Leu Thr Ser Gly Lys Leu Tyr Tyr Glu Leu Ala
1115 1120 1125

Ala Arg Lys Ala Lys Asp Asn Arg Asn Asp Leu Ala Ile Val Arg
1130 1135 1140

Leu Glu Gln Leu Ala Pro Leu Pro Arg Arg Arg Leu Arg Glu Thr
1145 1150 1155

Leu Asp Arg Tyr Glu Asn Val Lys Glu Phe Phe Trp Val Gln Glu
1160 1165 1170

Glu Pro Ala Asn Gln Gly Ala Trp Pro Arg Phe Gly Leu Glu Leu
1175 1180 1185

Pro Glu Leu Leu Pro Asp Lys Leu Ala Gly Ile Lys Arg Ile Ser
1190 1195 1200

Arg Arg Ala Met Ser Ala Pro Ser Ser Gly Ser Ser Lys Val His
1205 1210 1215

Ala Val Glu Gln Gln Glu Ile Leu Asp Glu Ala Phe Gly
1220 1225 1230

<210> 47
<211> 3696
<212> DNA
<213> 人造

<220>
<223> 結核分枝桿菌α酮戊二酸去羧苷 KgD 密碼子最佳化基因

<400> 47

atggctaata tctcctctcc gtttggtcag aatgaatggc tggtagaaga aatgtaccgt 60

aaattccgcg atgacccgtc ctctgtggac ccgtcctggc atgaattcct ggtagactac 120

agcccggagc cgaccagcca accggcagcg gaaccaaccc gcgttacttc tccgctggta 180

gcggaacgtg cagctgctgc cgcgcctcag gcgccgccta aaccggcgga tactgccgca 240

gccggtaacg gtgtggtggc cgcactggct gctaagactg cggttccgcc gccagcagaa 300

ggcgatgaag ttgcagtcct gcgcggtgcg gcggctgcag tggtgaaaaa catgagcgcg 360

tccctggagg taccgaccgc cacgagcgtg cgcgcggtcc ctgctaaact gctgattgat 420

aaccgtattg tgatcaacaa ccagctgaaa cgtacccgtg gtggcaagat ctccttcact 480

catctgctgg gttatgcact ggtacaagcg gttaagaaat tccctaacat gaaccgtcat 540

tacactgagg tcgacggtaa accgacggct gttactccgg cacacacgaa cctgggcctg 600

gcgatcgacc tgcaaggtaa agatggtaag cgctccctgg tagttgcggg tattaaacgt 660

tgcgaaacca tgcgtttcgc acaattcgta accgcctacg aggacattgt ccgccgtgct 720

cgtgatggca aactgaccac cgaagatttt gcgggcgtta ctattagcct gaccaaccca 780

ggcaccatcg gcaccgtgca cagcgtacct cgtctgatgc cgggccaagg tgcgattatc 840

ggtgtgggtg ccatggagta cccggcagaa tttcagggtg cttctgaaga gcgcatcgcc 900

gagctgggta ttggtaaact gatcaccctg acttctacct atgaccaccg catcattcag 960

ggcgcagaat ccggtgactt cctgcgcact attcacgaac tgctgctgtc cgacggtttc 1020

tgggatgaag tttttcgtga actgagcatc ccatatctgc cagttcgctg gtccaccgac 1080

aatccggact ctatcgttga caaaaacgct cgcgtaatga acctgatcgc tgcttatcgt 1140

aatcgtggtc acctgatggc tgatacggat ccgctgcgcc tggataaagc tcgtttccgt 1200

tcccacccgg acctggaagt gctgacccat ggtctgactc tgtgggatct ggaccgcgtg 1260

ttcaaagtag atggtttcgc gggtgctcag tacaagaagc tgcgtgacgt gctgggtctg 1320

ctgcgtgatg cgtactgtcg tcacattggt gtggagtacg cccacattct ggatccggaa 1380

cagaaagaat ggctggagca gcgtgtcgag accaaacacg taaaaccgac cgtagcgcag 1440

cagaaatata tcctgtccaa actgaacgcc gccgaggctt tcgaaacttt cctgcagacc 1500

aagtacgtgg gccagaaacg cttcagcctg gagggtgcgg aaagcgttat tccgatgatg 1560

gatgcagcta tcgatcagtg cgcggaacat ggtctggatg aagtcgttat cggtatgccg 1620

caccgtggtc gcctgaacgt actggcaaac atcgtcggta aaccatattc tcagatcttc 1680

acggaattcg agggcaacct gaacccgtcc caagcccacg gctccggcga cgtaaaatat 1740

catctgggtg ctaccggcct gtatctgcag atgttcggtg ataacgacat ccaggtatct 1800

ctgactgcta acccgagcca cctggaggcg gttgatcctg ttctggaagg tctggttcgc 1860

gccaaacagg atctgctgga ccacggctct atcgacagcg atggccagcg tgcattcagc 1920

gttgtaccgc tgatgctgca tggcgacgcg gcgttcgccg gtcagggtgt cgtagcagaa 1980

actctgaacc tggcgaacct gcctggctat cgcgtgggtg gcaccattca catcatcgtt 2040

aacaaccaaa tcggtttcac cacggcaccg gagtatagcc gttctagcga atattgcacc 2100

gacgtagcca aaatgatcgg tgcgccgatc ttccatgtaa acggtgacga tccagaggcc 2160

tgcgtgtggg tggctcgtct ggccgtagac ttccgccagc gttttaagaa agatgtggtt 2220

atcgacatgc tgtgctaccg ccgtcgtggt cacaacgaag gtgatgatcc gtctatgact 2280

aacccgtatg tctatgacgt ggtggacacc aagcgtggtg cacgcaaatc ttacacggag 2340

gccctgatcg gtcgtggcga catctctatg aaagaagcgg aagacgctct gcgtgattac 2400

cagggtcagc tggaacgtgt gttcaatgag gtgcgtgagc tggaaaagca cggcgtacaa 2460

ccgtccgaat ccgtagagtc cgatcagatg atccctgctg gtctggcaac tgctgttgat 2520

aaaagcctgc tggcgcgtat cggcgacgca ttcctggcgc tgccgaatgg ctttaccgcg 2580

cacccgcgcg tacagccggt actggaaaaa cgtcgtgaaa tggcctacga aggtaaaatc 2640

gattgggcct tcggtgagct gctggccctg ggctctctgg tggctgaggg caagctggta 2700

cgcctgagcg gccaggactc ccgtcgcggc acttttctc agcgtcacag cgtcctgatc 2760

gatcgtcaca ccggcgaaga attcacgccg ctgcaactgc tggctactaa ctccgatggt 2820

agcccgaccg gtggtaagtt cctggtgtac gattccccgc tgtccgaata tgctgcagtt 2880

ggtttcgagt atggttacac cgttggcaac ccggacgcag tggttctgtg ggaagcgcag 2940

ttcggcgatt tcgttaacgg tgcccagtcc attatcgatg agtttattag cagcggcgag 3000

gccaaatggg gccagctgtc taacgttgtg ctgctgctgc ctcacggcca cgagggtcaa 3060

ggcccggacc acacctccgc ccgtatcgaa cgcttcctgc agctgtgggc tgaaggctct 3120

atgaccatcg cgatgccgtc taccccaagc aactacttcc acctgctgcg tcgccacgca 3180

ctggacggca ttcagcgccc gctgatcgtt ttcaccccaa aatccatgct gcgccacaaa 3240

gcagctgttt ctgaaatcaa agattttacg gaaattaaat tccgttctgt gctggaagaa 3300

ccaacctacg aagacggtat tggcgaccgc aacaaggtaa gccgtatcct gctgacctcc 3360

ggcaaactgt actacgagct ggcagcacgt aaggcaaaag ataaccgcaa cgacctggcc 3420

atcgtccgcc tggaacagct ggcgccactg ccacgccgtc gcctgcgtga aaccctggat 3480

cgctacgaaa acgtaaaaga attcttctgg gtgcaggaag aaccggcaaa ccagggtgcg 3540

tggccgcgct ttggtctgga actgccggaa ctgctgccgg ataaactggc aggtatcaag 3600

cgcatcagcc gtcgcgctat gagcgccccg tcttctggta gctctaaagt acacgctgta 3660

gaacagcaag agatcctgga tgaggccttc ggctaa 3696

<210> 48
<211> 74
<212> DNA
<213> 人造序列

<220>
<223> 用於枯草桿菌轉胺苷 x 之擴增之正錄引子

<400> 48
ggggacaagt ttgtacaaaa aagcaggcta ggaggaatta accatgaagg ttttagtcaa 60

tggccggctg attg 74

<210> 49
<211> 62
<212> DNA
<213> 人造序列

<220>
<223> 用於枯草桿菌轉胺苷 x 之擴增之反錄引子

<400> 49
ggggaccact ttgtacaaga aagctgggtt tatgaaatgc tagcagcctg ttgaatgctt 60

tc 62

<210> 50
<211> 82
<212> DNA
<213> 人造序列

<220>
<223> 用於枯草桿菌轉胺苷 y 之擴增之正錄引子

<400> 50
ggggacaagt ttgtacaaaa aagcaggcta ggaggaatta accatgactc atgatttgat 60

agaaaaaagt aaaaagcacc tc 82

<210> 51
<211> 57
<212> DNA
<213> 人造序列

<220>
<223> 用於枯草桿菌轉胺苷 y 之擴增之反錄引子

<400> 51
ggggaccact ttgtacaaga aagctgggtt caatcttcaa ggctcgtaac ctcgtgg 57

<210> 52
<211> 64
<212> DNA
<213> 人造序列

<220>
<223> 用於球狀紅桿菌轉胺苷之擴增之正錄引子

<400> 52
ggggacaagt ttgtacaaaa aagcaggcta ggaggaatta accatgcccg gttgcggggg 60

cttg 64

<210> 53
<211> 51
<212> DNA
<213> 人造序列

<220>
<223> 用於球狀紅桿菌轉胺苷之擴增之反錄引子

<400> 53
ggggaccact ttgtacaaga aagctgggtt cagacggcgg ccggttcttt c 51

<210> 54
<211> 78
<212> DNA
<213> 人造序列

<220>
<223> 用於嗜肺性退伍軍人症菌轉胺苷之擴增之正錄引子

<400> 54
ggggacaagt ttgtacaaaa aagcaggcta ggaggaatta accatgagta tcgcatttgt 60

taacggcaag tattgttg 78

<210> 55
<211> 67
<212> DNA
<213> 人造序列

<220>
<223> 用於嗜肺性退伍軍人症菌轉胺苷之擴增之反錄引子

<400> 55
ggggaccact ttgtacaaga aagctgggtt tagtttacta gttgttggta ggaatcatta 60

attatcc 67

<210> 56
<211> 76
<212> DNA

<213> 人造序列

<220>
<223> 用於歐洲亞硝酸菌轉胺苷之擴增之正錄引子

<400> 56
ggggacaagt ttgtacaaaa aagcaggcta ggaggaatta accatgattt acctcaatgg 60

caaatttctg ccgatg 76

<210> 57
<211> 50
<212> DNA
<213> 人造序列

<220>
<223> 用於歐洲亞硝酸菌轉胺苷之擴增之反錄引子

<400> 57
ggggaccact ttgtacaaga aagctgggtt tactggcgtg gagcatgccc 50

<210> 58
<211> 79
<212> DNA
<213> 人造序列

<220>
<223> 用於淋病奈瑟氏菌轉胺苷之擴增之正錄引子

<400> 58
ggggacaagt ttgtacaaaa aagcaggcta ggaggaatta accatgagga taaatatgaa 60

ccgtaacgaa attttattc 79

<210> 59
<211> 56
<212> DNA
<213> 人造序列

<220>
<223> 用於淋病奈瑟氏菌轉胺苷之擴增之反錄引子

<400> 59
ggggaccact ttgtacaaga aagctgggtt catgcagcca tcgccttgaa cacttc 56

<210> 60
<211> 66
<212> DNA
<213> 人造序列

<220>
<223> 用於綠膿桿菌轉胺苷之擴增之正錄引子

<400> 60
ggggacaagt ttgtacaaaa aagcaggcta ggaggaatta accatgtcga tggccgatcg 60

tgatgg 66

<210> 61
<211> 53
<212> DNA
<213> 人造序列

<220>
<223> 用於綠膿桿菌轉胺苷之擴增之反錄引子

<400> 61
ggggaccact ttgtacaaga aagctgggtt tacttgacca gggtacgcca ctc 53

<210> 62
<211> 67
<212> DNA
<213> 人造序列

<220>
<223> 用於沼澤紅假單胞菌轉胺苷之擴增之正錄引子

<400> 62
ggggacaagt ttgtacaaaa aagcaggcta ggaggaatta accatgaagc tgataccgtg 60

ccgcgcc 67

<210> 63
<211> 51
<212> DNA
<213> 人造序列

<220>
<223> 用於沼澤紅假單胞菌轉胺苷之擴增之反錄引子
aminotransferase

<400> 63
ggggaccact ttgtacaaga aagctgggtt caggcgaccg cgcggatcac c 51

<210> 64
<211> 1353
<212> DNA
<213> 枯草桿菌轉胺苷基因(gi16077991)

<400> 64
atggagatga tggggatgga aaacattcag caaaatcagg gattaaagca aaaagatgag 60

caatttgtgt ggcatgccat gaagggagcg catcaagcgg acagcctgat agcccagaag 120

gccgaagggg cctgggtaac cgacacagac ggacgccgct atttggatgc gatgtccggt 180

ttgtggtgcg tcaacattgg ttacggcaga aaggagcttg cggaggctgc ctatgagcaa 240

ctaaaggagc tgccttacta cccgttaacg caaagtcacg cacccgcaat tcaactggcg 300

gaaaagctga atgaatggct tggcggcgat tatgttattt ttttttccaa cagcggatcg 360

gaagcaaacg aaactgcttt taaaattgcc cgccagtacc atctgcaaaa cggcgaccac 420

agccgttata aattcatctc aagatatcgg gcataccacg gcaatacatt gggagcgctc 480

tcagctaccg gacaggcgca gcggaaatat aaatacgagc ctttgagcca agggttcctg 540

catgcagctc cgccagatat ataccggaat cctgatgatg cagacacgct tgaaagcgca 600

aatgaaatcg accgcatcat gacatgggaa ttaagcgaaa cgattgccgg ggtcattatg 660

gagcccatca ttacaggcgg aggcatccta atgccgccgg acggatatat gaagaaggtg 720

gaggacattt gccggcgcca cggagccctt ttgatttgcg atgaagtgat ctgcgggttt 780

ggacggacag gtgagccgtt cgggtttatg cactacggtg tgaagcctga tatcattacg 840

atggcaaagg gaatcacaag cgcgtatctg ccattgtcag cgactgctgt gaaacgggac 900

attttcgaag cgtatcaggg ggaagctcct tatgaccgtt tccgccacgt gaacacgttc 960

ggcggaagcc cggctgcctg tgctttggcg ttgaaaaacc tgcaaattat ggaggacgaa 1020

cagctgattc agcgatcccg tgatcttgga gcaaagcttt taggtgagct tcaagctctg 1080

agagaacacc cggcagtcgg ggatgttaga ggaaaagggc tgctgatcgg aatcgaactc 1140

gtcaaagaca aattgactaa agagccggct gatgccgcca aagtaaacca agtggttgcg 1200

gcgtgcaaag aaaaagggct gatcatcggc aaaaacggcg atacagtcgc cggctacaac 1260

aatgtcatcc acgttgcgcc gccattttgc ctgacagaag aggacctttc ctttatcgtg 1320

aaaacggtga aagaaagctt tcaaacgata taa 1353

<210> 65
<211> 450
<212> PRT
<213> 枯草桿菌轉胺苷(gi16077991)之胺基酸序列

<400> 65

Met Glu Met Met Gly Met Glu Asn Ile Gln Gln Asn Gln Gly Leu Lys
1 5 10 15

Gln Lys Asp Glu Gln Phe Val Trp His Ala Met Lys Gly Ala His Gln
20 25 30

Ala Asp Ser Leu Ile Ala Gln Lys Ala Glu Gly Ala Trp Val Thr Asp
35 40 45

Thr Asp Gly Arg Arg Tyr Leu Asp Ala Met Ser Gly Leu Trp Cys Val
50 55 60

Asn Ile Gly Tyr Gly Arg Lys Glu Leu Ala Glu Ala Ala Tyr Glu Gln
65 70 75 80

Leu Lys Glu Leu Pro Tyr Tyr Pro Leu Thr Gln Ser His Ala Pro Ala
85 90 95

Ile Gln Leu Ala Glu Lys Leu Asn Glu Trp Leu Gly Gly Asp Tyr Val
100 105 110

Ile Phe Phe Ser Asn Ser Gly Ser Glu Ala Asn Glu Thr Ala Phe Lys
115 120 125

Ile Ala Arg Gln Tyr His Leu Gln Asn Gly Asp His Ser Arg Tyr Lys
130 135 140

Phe Ile Ser Arg Tyr Arg Ala Tyr His Gly Asn Thr Leu Gly Ala Leu
145 150 155 160

Ser Ala Thr Gly Gln Ala Gln Arg Lys Tyr Lys Tyr Glu Pro Leu Ser
165 170 175

Gln Gly Phe Leu His Ala Ala Pro Pro Asp Ile Tyr Arg Asn Pro Asp
180 185 190

Asp Ala Asp Thr Leu Glu Ser Ala Asn Glu Ile Asp Arg Ile Met Thr
195 200 205

Trp Glu Leu Ser Glu Thr Ile Ala Gly Val Ile Met Glu Pro Ile Ile
210 215 220

Thr Gly Gly Gly Ile Leu Met Pro Pro Asp Gly Tyr Met Lys Lys Val
225 230 235 240

Glu Asp Ile Cys Arg Arg His Gly Ala Leu Leu Ile Cys Asp Glu Val
245 250 255

Ile Cys Gly Phe Gly Arg Thr Gly Glu Pro Phe Gly Phe Met His Tyr
260 265 270

Gly Val Lys Pro Asp Ile Ile Thr Met Ala Lys Gly Ile Thr Ser Ala
275 280 285

Tyr Leu Pro Leu Ser Ala Thr Ala Val Lys Arg Asp Ile Phe Glu Ala
290 295 300

Tyr Gln Gly Glu Ala Pro Tyr Asp Arg Phe Arg His Val Asn Thr Phe
305 310 315 320

Gly Gly Ser Pro Ala Ala Cys Ala Leu Ala Leu Lys Asn Leu Gln Ile
325 330 335

Met Glu Asp Glu Gln Leu Ile Gln Arg Ser Arg Asp Leu Gly Ala Lys
340 345 350

Leu Leu Gly Glu Leu Gln Ala Leu Arg Glu His Pro Ala Val Gly Asp
355 360 365

Val Arg Gly Lys Gly Leu Leu Ile Gly Ile Glu Leu Val Lys Asp Lys
370 375 380

Leu Thr Lys Glu Pro Ala Asp Ala Ala Lys Val Asn Gln Val Val Ala
385 390 395 400

Ala Cys Lys Glu Lys Gly Leu Ile Ile Gly Lys Asn Gly Asp Thr Val
405 410 415

Ala Gly Tyr Asn Asn Val Ile His Val Ala Pro Pro Phe Cys Leu Thr
420 425 430

Glu Glu Asp Leu Ser Phe Ile Val Lys Thr Val Lys Glu Ser Phe Gln
435 440 445

Thr Ile
450

<210> 66
<211> 1407
<212> DNA
<213> 綠膿桿菌轉胺苷基因(seq ID gi9951072)

<400> 66
atgaacgcaa gactgcacgc cacgtccccc ctcggcgacg ccgacctggt ccgtgccgac 60

caggcccact acatgcacgg ctaccacgtg ttcgacgacc accgcgtcaa cggctcgctg 120

aacatcgccg ccggcgacgg cgcctatatc tacgacaccg ccggcaaccg ctacctcgac 180

gcggtgggcg gcatgtggtg caccaacatc ggcctggggc gcgaggaaat ggctcgcacc 240

gtggccgagc agacccgcct gctggcctat tccaatccct tctgcgacat ggccaacccg 300

cgcgccatcg aactctgccg caagctcgcc gagctggccc ccggcgacct cgaccacgtg 360

ttcctcacca ccggcggttc caccgccgtg gacaccgcga tccgcctcat gcactactac 420

cagaactgcc gcggcaagcg cgccaagaag cacgtcatca cgcggatcaa cgcctaccac 480

ggctcgacct tcctcggcat gtcgctgggc ggcaagagcg ccgaccggcc ggccgagttc 540

gacttcctcg acgagcgcat ccaccacctc gcctgtccct attactaccg cgctccggaa 600

gggctgggcg aagccgagtt cctcgatggc ctggtggacg agttcgaacg caagatcctc 660

gaactgggcg ccgaccgggt gggggcgttc atctccgagc cggtgttcgg ctccggcggc 720

gtgatcgtcc cgcccgcggg ctaccacagg cggatgtggg agctgtgcca gcgctacgac 780

gtgctgtaca tctccgacga agtggtgacc tccttcggcc gcctcggcca cttcttcgcc 840

agccaggcgg tgttcggcgt acagccggac atcatcctca ccgccaaggg cctcacctcc 900

ggctaccagc cgctgggcgc gtgcatcttc tcccggcgca tctgggaggt gatcgccgag 960

ccggacaagg gccgctgctt cagccatggt ttcacctact ccggccaccc ggtggcctgc 1020

gcggcggcgc tgaagaacat cgagatcatc gagcgcgagg gcttgctcgc ccacgccgac 1080

gaggtcggcc gctacttcga ggagcgcctg caaagcctcc gcgacctgcc catcgtcggc 1140

gacgtgcgcg ggatgcgctt catggcctgt gtcgagttcg tcgccgacaa ggcgagcaag 1200

gcgctgtttc cggaaagcct gaacatcggc gagtgggtcc acctgcgggc gcagaagcgc 1260

ggcctgctgg ttcgtccgat cgtccacctg aacgtgatgt cgccgccgct gatcctcacc 1320

cgcgaacagg tcgataccgt ggtccgggtg ctgcgcgaga gcatcgagga aaccgtggag 1380

gatcttgtcc gcgccggtca ccggtaa 1407

<210> 67
<211> 468
<212> PRT
<213> 綠膿桿菌轉胺苷(seq ID gi9951072)之胺基酸序列

<400> 67

Met Asn Ala Arg Leu His Ala Thr Ser Pro Leu Gly Asp Ala Asp Leu
1 5 10 15

Val Arg Ala Asp Gln Ala His Tyr Met His Gly Tyr His Val Phe Asp
20 25 30

Asp His Arg Val Asn Gly Ser Leu Asn Ile Ala Ala Gly Asp Gly Ala
35 40 45

Tyr Ile Tyr Asp Thr Ala Gly Asn Arg Tyr Leu Asp Ala Val Gly Gly
50 55 60

Met Trp Cys Thr Asn Ile Gly Leu Gly Arg Glu Glu Met Ala Arg Thr
65 70 75 80

Val Ala Glu Gln Thr Arg Leu Leu Ala Tyr Ser Asn Pro Phe Cys Asp
85 90 95

Met Ala Asn Pro Arg Ala Ile Glu Leu Cys Arg Lys Leu Ala Glu Leu
100 105 110

Ala Pro Gly Asp Leu Asp His Val Phe Leu Thr Thr Gly Gly Ser Thr
115 120 125

Ala Val Asp Thr Ala Ile Arg Leu Met His Tyr Tyr Gln Asn Cys Arg
130 135 140

Gly Lys Arg Ala Lys Lys His Val Ile Thr Arg Ile Asn Ala Tyr His
145 150 155 160

Gly Ser Thr Phe Leu Gly Met Ser Leu Gly Gly Lys Ser Ala Asp Arg
165 170 175

Pro Ala Glu Phe Asp Phe Leu Asp Glu Arg Ile His His Leu Ala Cys
180 185 190

Pro Tyr Tyr Tyr Arg Ala Pro Glu Gly Leu Gly Glu Ala Glu Phe Leu
195 200 205

Asp Gly Leu Val Asp Glu Phe Glu Arg Lys Ile Leu Glu Leu Gly Ala
210 215 220

Asp Arg Val Gly Ala Phe Ile Ser Glu Pro Val Phe Gly Ser Gly Gly
225 230 235 240

Val Ile Val Pro Pro Ala Gly Tyr His Arg Arg Met Trp Glu Leu Cys
245 250 255

Gln Arg Tyr Asp Val Leu Tyr Ile Ser Asp Glu Val Val Thr Ser Phe
260 265 270

Gly Arg Leu Gly His Phe Phe Ala Ser Gln Ala Val Phe Gly Val Gln
275 280 285

Pro Asp Ile Ile Leu Thr Ala Lys Gly Leu Thr Ser Gly Tyr Gln Pro

290 295 300

Leu Gly Ala Cys Ile Phe Ser Arg Arg Ile Trp Glu Val Ile Ala Glu
305 310 315 320

Pro Asp Lys Gly Arg Cys Phe Ser His Gly Phe Thr Tyr Ser Gly His
325 330 335

Pro Val Ala Cys Ala Ala Ala Leu Lys Asn Ile Glu Ile Ile Glu Arg
340 345 350

Glu Gly Leu Leu Ala His Ala Asp Glu Val Gly Arg Tyr Phe Glu Glu
355 360 365

Arg Leu Gln Ser Leu Arg Asp Leu Pro Ile Val Gly Asp Val Arg Gly
370 375 380

Met Arg Phe Met Ala Cys Val Glu Phe Val Ala Asp Lys Ala Ser Lys
385 390 395 400

Ala Leu Phe Pro Glu Ser Leu Asn Ile Gly Glu Trp Val His Leu Arg
405 410 415

Ala Gln Lys Arg Gly Leu Leu Val Arg Pro Ile Val His Leu Asn Val
420 425 430

Met Ser Pro Pro Leu Ile Leu Thr Arg Glu Gln Val Asp Thr Val Val
435 440 445

Arg Val Leu Arg Glu Ser Ile Glu Glu Thr Val Glu Asp Leu Val Arg
450 455 460

Ala Gly His Arg
465

<210> 68
<211> 1335
<212> DNA
<213> 綠膿桿菌轉胺苷基因(seq ID gi9951630)

<400> 68
atgacaatga atgacgagcc gcagtcgagc agcctcgaca acttctggat gcccttcacc 60

gccaaccgcc agttcaaggc gcggccgcgc ctgctggaaa gcgccgaagg catccactat 120

atcgcccagg gcgggcgccg catcctcgac ggcaccgccg gcctctggtg ctgcaatgcc 180

ggccacggcc ggcgcgagat cagcgaagcg gtggcccggc agatcgccac cctcgactac 240

gccccgccgt tccagatggg tcacccgctg ccgttcgaac tcgccgcgcg gctgacggaa 300

atcgccccgc cgagcctgaa caaagtattc ttcaccaact ccggctcgga atcggcggac 360

accgcgctga agatcgccct tgcctaccag cgcgccatcg gccagggcac ccgcacccgc 420

ctgatcggcc gcgaactggg ctaccacggg gtcggcttcg gcggcctgtc ggtaggcggt 480

atggtcaaca accgcaaggc cttctccgcc aacctgctgc cgggggtcga ccacctgccg 540

cacaccctgg acgtcgcccg caacgccttc accgtcggcc tgcccgagca tggcgtggaa 600

aaggccgagg agctggaacg cctggtgacc ctgcacggcg ccgagaatat cgccgcggtg 660

atcgtcgagc cgatgtccgg ctcggccggc gtggtgctgc cgcccaaggg ctaccttcag 720

cggctgcgcg agataacccg caagcatggc atcctgctga tcttcgacga agtgatcacc 780

ggtttcggcc gcgtcggcga agccttcgcc gcgcagcgct ggggcgtcgt cccggacctg 840

ctgacctgcg ccaaggggct gaccaacggc agcatcccga tgggcgccgt attcgtcgac 900

gagaagatcc atgctgcctt catgcaaggc ccgcagggcg ccatcgagtt cttccacggc 960

tatacctatt ccggccatcc ggtagcctgc gccgccgccc tggcgaccct ggacatctac 1020

cgtcgcgacg acctgttcca gcgggccgtc gaactggaag gctactggca ggacgcgctg 1080

ttcagcctgc gcgacctgcc caacgtggtc gacatccgcg ccgtaggcct ggtcggcggc 1140

gtgcaactgg cgccgcacgc ggacggcccc ggcaagcgcg gctacgacgt cttcgagcgc 1200

tgcttctggg agcacgacct gatggtccgg gtgaccggcg acatcatcgc catgtcgccg 1260

ccgctgatca tcgacaagcc ccacatcgac cagatcgtcg agcgcctggc ccaggccatc 1320

cgcgccagcg tctga 1335

<210> 69
<211> 444
<212> PRT
<213> 綠膿桿菌轉胺苷(seq ID gi9951630)之胺基酸序列

<400> 69

Met Thr Met Asn Asp Glu Pro Gln Ser Ser Ser Leu Asp Asn Phe Trp
1 5 10 15

Met Pro Phe Thr Ala Asn Arg Gln Phe Lys Ala Arg Pro Arg Leu Leu
20 25 30

Glu Ser Ala Glu Gly Ile His Tyr Ile Ala Gln Gly Gly Arg Arg Ile
35 40 45

Leu Asp Gly Thr Ala Gly Leu Trp Cys Cys Asn Ala Gly His Gly Arg
50 55 60

Arg Glu Ile Ser Glu Ala Val Ala Arg Gln Ile Ala Thr Leu Asp Tyr
65 70 75 80

Ala Pro Pro Phe Gln Met Gly His Pro Leu Pro Phe Glu Leu Ala Ala
85 90 95

Arg Leu Thr Glu Ile Ala Pro Pro Ser Leu Asn Lys Val Phe Phe Thr
100 105 110

Asn Ser Gly Ser Glu Ser Ala Asp Thr Ala Leu Lys Ile Ala Leu Ala
115 120 125

Tyr Gln Arg Ala Ile Gly Gln Gly Thr Arg Thr Arg Leu Ile Gly Arg
130 135 140

Glu Leu Gly Tyr His Gly Val Gly Phe Gly Gly Leu Ser Val Gly Gly
145 150 155 160

Met Val Asn Asn Arg Lys Ala Phe Ser Ala Asn Leu Leu Pro Gly Val
165 170 175

Asp His Leu Pro His Thr Leu Asp Val Ala Arg Asn Ala Phe Thr Val
180 185 190

Gly Leu Pro Glu His Gly Val Glu Lys Ala Glu Glu Leu Glu Arg Leu
195 200 205

Val Thr Leu His Gly Ala Glu Asn Ile Ala Ala Val Ile Val Glu Pro
210 215 220

Met Ser Gly Ser Ala Gly Val Val Leu Pro Pro Lys Gly Tyr Leu Gln
225 230 235 240

Arg Leu Arg Glu Ile Thr Arg Lys His Gly Ile Leu Leu Ile Phe Asp
245 250 255

Glu Val Ile Thr Gly Phe Gly Arg Val Gly Glu Ala Phe Ala Ala Gln
260 265 270

Arg Trp Gly Val Val Pro Asp Leu Leu Thr Cys Ala Lys Gly Leu Thr
275 280 285

Asn Gly Ser Ile Pro Met Gly Ala Val Phe Val Asp Glu Lys Ile His
290 295 300

Ala Ala Phe Met Gln Gly Pro Gln Gly Ala Ile Glu Phe Phe His Gly
305 310 315 320

Tyr Thr Tyr Ser Gly His Pro Val Ala Cys Ala Ala Ala Leu Ala Thr
325 330 335

Leu Asp Ile Tyr Arg Arg Asp Asp Leu Phe Gln Arg Ala Val Glu Leu
340 345 350

Glu Gly Tyr Trp Gln Asp Ala Leu Phe Ser Leu Arg Asp Leu Pro Asn
355 360 365

Val Val Asp Ile Arg Ala Val Gly Leu Val Gly Gly Val Gln Leu Ala
370 375 380

Pro His Ala Asp Gly Pro Gly Lys Arg Gly Tyr Asp Val Phe Glu Arg
385 390 395 400

Cys Phe Trp Glu His Asp Leu Met Val Arg Val Thr Gly Asp Ile Ile
405 410 415

Ala Met Ser Pro Pro Leu Ile Ile Asp Lys Pro His Ile Asp Gln Ile
420 425 430

Val Glu Arg Leu Ala Gln Ala Ile Arg Ala Ser Val
435 440

<210> 70
<211> 71
<212> DNA
<213> 用於枯草桿菌轉胺苷(gi16077991)之擴增之正錄引子

<400> 70
ggggacaagt ttgtacaaaa aagcaggcta ggaggaatta accatggaga tgatggggat 60

ggaaaacatt c 71

<210> 71
<211> 65
<212> DNA
<213> 用於枯草桿菌轉胺苷(gi16077991)之擴增之反錄引子

<400> 71

ggggaccact ttgtacaaga aagctgggtt tatatcgttt gaaagctttc tttcaccgtt 60

ttcac 65

<210> 72
<211> 66
<212> DNA
<213> 用於綠膿桿菌轉胺苷(gi9951072)之擴增之正錄引子

<400> 72
ggggacaagt ttgtacaaaa aagcaggcta ggaggaatta accatgaacg caagactgca 60

cgccac 66

<210> 73
<211> 48
<212> DNA
<213> 用於綠膿桿菌轉胺苷(gi9951072)之擴增之反錄引子

<400> 73
ggggaccact ttgtacaaga aagctgggtt taccggtgac cggcgcgg 48

<210> 74
<211> 69
<212> DNA
<213> 用於綠膿桿菌轉胺苷(gi9951630)之擴增之正錄引子

<400> 74
ggggacaagt ttgtacaaaa aagcaggcta ggaggaatta accatgacaa tgaatgacga 60

gccgcagtc 69

<210> 75
<211> 49
<212> DNA
<213> 用於綠膿桿菌轉胺苷(gi9951630)之擴增之反錄引子

<400> 75
ggggaccact ttgtacaaga aagctgggtt cagacgctgg cgcggatgg 49

<210> 76
<211> 57
<212> DNA
<213> 人造序列

<220>
<223> 正錄引子

<400> 76
aaatttacta gtaagaattt ttgaggaggc aatataaatg aataaaccac agtcttg 57

<210> 77
<211> 32

<212> DNA
<213> 人造序列

<220>
<223> 反錄引子

<400> 77
aaatttggat cctacaagaa agctgggttt ac 32

<210> 78
<211> 58
<212> DNA
<213> 人造序列

<220>
<223> 正錄引子

<400> 78
aaatttacta gtaagaattt ttgaggaggc aatataaatg aacagccaaa tcaccaac 58

<210> 79
<211> 37
<212> DNA
<213> 人造序列

<220>
<223> 反錄引子

<400> 79
aaatttggat ccactttgta caagaaagct gggttca 37

<210> 80
<211> 57
<212> DNA
<213> 人造序列

<220>
<223> 正錄引子

<400> 80
aaatttggat ccgttgagga ggcctcaaaa atgtccgaga tcactctggg caaatac 57

<210> 81
<211> 35
<212> DNA
<213> 人造序列

<220>
<223> 反錄引子

<400> 81
aaatttggcg cgccattact gtttagcgtt agttg 35

<210> 82

<211> 52
<212> DNA
<213> 人造序列

<220>
<223> 正錄引子

<400> 82
aaatttggat ccgttgagga ggcctcaaaa atgtatactg ttggtgatta tc 52

<210> 83
<211> 37
<212> DNA
<213> 人造序列

<220>
<223> 反錄引子

<400> 83
aaatttggcg cgccattact tgttctgctc cgcaaac 37

3

105

105

I627153

公告本

發明摘要

※ 申請案號：104120521 (由98107957 分割)

※ 申請日：98.3.11 ※IPC 分類：C07C 229/08 (2006.01)
C12P 13/00 (2006.01)
C12P 17/10 (2006.01)

【發明名稱】(中文/英文)

由α-酮庚二酸製備6-胺己酸之技術

PREPARATION OF 6-AMINOCAPROIC ACID FROM α-KETOPIMELIC ACID

【中文】

本發明係關於一種使用生物催化劑製備6-胺己酸(後文也稱作爲「6-ACA」)之方法。本發明進一步係關於一種經由環化此種6-ACA製備ε-己內醯胺(後文稱作爲「己內醯胺」)之方法。本發明進一步係關於可用於6-ACA或己內醯胺之製備之宿主細胞、微生物、或多核苷酸。

【英文】

The invention relates to a method for preparing 6-aminocaproic acid (hereinafter also referred to as '6-ACA') using a biocatalyst. The invention further relates to a method for preparing ε-caprolactam (hereafter referred to as 'caprolactam') by cyclising such 6-ACA. The invention further relates to a host cell, a micro-organism, or a polynucleotide which may be used in the preparation of 6-ACA or caprolactam.

【代表圖】

【本案指定代表圖】：第（　　）圖。(無)

【本代表圖之符號簡單說明】：

【本案若有化學式時，請揭示最能顯示發明特徵的化學式】：

(無)

公告本

申請專利範圍

107年3月26日 修正 補充

1. 一種用於製備6-胺己酸之方法，其中該6-胺己酸係使用一微生物而由5-甲醯戊酸鹽製備，其中該微生物包含可催化轉胺化反應或還原胺化反應之酶，以由5-甲醯戊酸鹽產生6-胺己酸，其中該可催化轉胺化反應之酶是選自轉胺酶(E.C. 2.6.1)之組群，且其中該可催化還原胺化反應之酶是選自胺基酸去氫酶(E.C.1.4.1)之組群。
2. 如請求項1之方法，其中該微生物包含該可催化轉胺化反應之酶以及該可催化還原胺化反應之酶。
3. 如請求項1或2之方法，其中該可催化轉胺化反應之酶係選自於由β-胺異丁酸:α-酮戊二酸轉胺酶、β-丙胺酸轉胺酶、天冬酸轉胺酶、4-胺丁酸轉胺酶(EC 2.6.1.19)、L-離胺酸6-轉胺酶(EC 2.6.1.36)、2-胺己二酸轉胺酶(EC 2.6.1.39)、5-胺戊酸轉胺酶(EC 2.6.1.48)、2-胺己酸轉胺酶(EC 2.6.1.67)及離胺酸:丙酮酸6-轉胺酶(EC 2.6.1.71)所組成之組群。
4. 如請求項1或2之方法，其中該可催化還原胺化反應之酶係離胺酸-6-去氫酶(EC 1.4.1.18)。
5. 如請求項1或2之方法，其中該可催化轉胺化反應及/或還原胺化反應之酶係得自選自於由下列所組成之組群之有機體：弧菌屬(*Vibrio*)；假單胞菌屬(*Pseudomonas*)；芽胞桿菌屬(*Bacillus*)；山靛屬(*Mercurialis*)；鐵角蕨屬(*Asplenium*)；佳樂樹屬(*Ceratonia*)；哺乳動物；紅黴屬

(*Neurospora*)；埃希氏菌屬(*Escherichia*)；棲熱菌屬(*Thermus*)；酵母屬(*Saccharomyces*)；短桿菌屬(*Brevibacterium*)；棒桿菌屬(*Corynebacterium*)；變形桿菌屬(*Proteus*)；土壤桿菌屬(*Agrobacterium*)；地桿菌屬(*Geobacillus*)；不動桿菌屬(*Acinetobacter*)；拉斯東氏菌屬(*Ralstonia*)；沙門氏菌屬(*Salmonella*)；紅細菌屬(*Rhodobacter*)及葡萄球菌屬(*Staphylococcus*)。

6. 如請求項1或2之方法，其中該可催化轉胺化反應及/或還原胺化反應之酶係得自選自於由下列所組成之組群之有機體：枯草桿菌(*Bacillus subtilis*)、韋氏芽胞桿菌(*Bacillus weihenstephanensis*)、球狀紅細菌(*Rhodobacter sphaeroides*)、金黃葡萄球菌(*Staphylococcus aureus*)、嗜肺性退伍軍人症桿菌(*Legionella pneumophilia*)、歐洲亞硝酸菌(*Nitrosomas europaea*)、淋病奈瑟氏菌(*Neisseria gonorrhoeae*)、環狀假單胞菌(*Pseudomonas syringae*)、沼澤紅假單胞菌(*Rhodopseudomonas palustris*)、河流弧菌(*Vibrio fluvialis*)及綠膿桿菌(*Pseudomonas aeruginosa*)。

7. 如請求項1或2之方法，其中該可催化轉胺化反應之酶是由根據序列ID 2、序列ID 5、序列ID 8、序列ID 12、序列ID 15、序列ID 17、序列ID 19、序列ID 21、序列ID 23、序列ID 25、序列ID 27、序列ID 29、序列ID 65、序列ID 67，或序列ID 69之胺基酸序列所構成。

8. 如請求項1之方法，其中該微生物係細菌、酵母或真菌。

9. 如請求項8之方法，其中該微生物係細菌。

10. 如請求項9之方法，其中該細菌係大腸桿菌(*Escherichia coli*)。

11. 如請求項8之方法，其中該微生物係酵母。

12. 如請求項11之方法，其中該酵母係釀酒酵母(*Saccharomyces cerevisiae*)。