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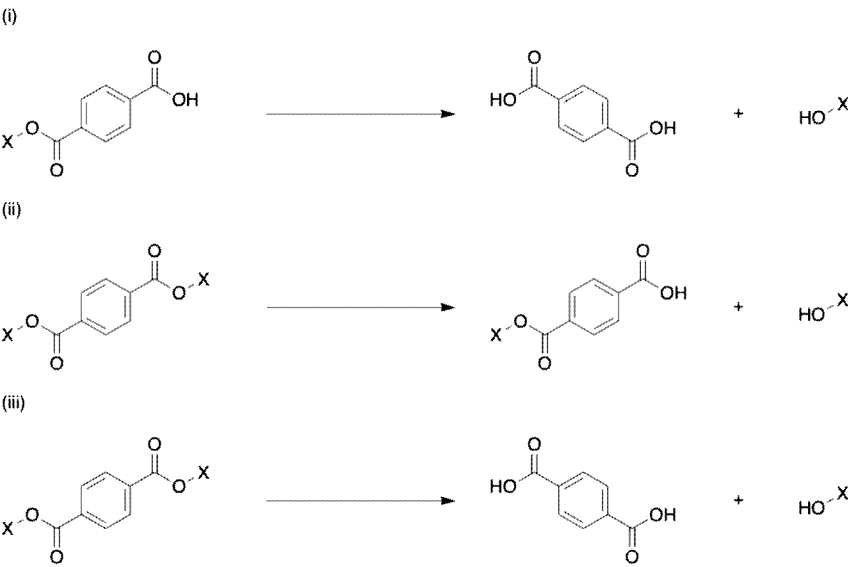
(54)名稱
酵素及其用途

(57)摘要

本發明大體上係關於具有酯酶活性之多肽，其中該酯酶活性能夠將對苯二甲酸單酯轉化成對苯二甲酸及醇；將對苯二甲酸二酯轉化成對苯二甲酸單酯及醇；或將對苯二甲酸二酯轉化成對苯二甲酸及醇，及其使用方法。

The present disclosure relates generally to polypeptides having esterase activity, wherein the esterase activity is capable of converting a monoester terephthalate to terephthalic acid and an alcohol; diester terephthalate to a monoester terephthalate and an alcohol; or diester terephthalate to terephthalic acid and an alcohol, and their methods of use.

指定代表圖：



【發明摘要】

【中文發明名稱】

酵素及其用途

【英文發明名稱】

ENZYMES AND USES THEREOF

【中文】

本發明大體上係關於具有酯酶活性之多肽，其中該酯酶活性能夠將對苯二甲酸單酯轉化成對苯二甲酸及醇；將對苯二甲酸二酯轉化成對苯二甲酸單酯及醇；或將對苯二甲酸二酯轉化成對苯二甲酸及醇，及其使用方法。

【英文】

The present disclosure relates generally to polypeptides having esterase activity, wherein the esterase activity is capable of converting a monoester terephthalate to terephthalic acid and an alcohol; diester terephthalate to a monoester terephthalate and an alcohol; or diester terephthalate to terephthalic acid and an alcohol, and their methods of use.

【指定代表圖】

圖1

【代表圖之符號簡單說明】

無

【發明說明書】

【中文發明名稱】

酵素及其用途

【英文發明名稱】

ENZYMES AND USES THEREOF

【技術領域】

【0001】 本發明係關於新穎合成酵素，更特定言之係關於催化對苯二甲酸單-酯及對苯二甲酸二-酯之水解的重組酵素及其用途。

【先前技術】

【0002】 全部參考，包括本說明書中所引用之任何專利或專利申請案特此以引用之方式併入，以使得能夠完全理解本發明。儘管如此，在澳大利亞或在任何其他國家，此類參考不應理解為構成對該等文件中之任一者形成此項技術中之公共常識之部分的許可。

【0003】 全球工業化已具有顯著環境影響，其中尤其為塑膠及塑膠產品之製造及依賴性增加。儘管不斷努力地尋找塑膠之適合及環境可持續替代物(包括塑膠之製造及處置)，但此類產品仍為顯著問題且造成絕大部分環境污染物。此問題之主要促成因素之一為聚對苯二甲酸乙二酯(PET)及其廢料產品，全球每年產生數百萬噸該等產品。此問題之環境意義至少部分歸因於塑膠，特別係基於PET之產品之化學性質，因為該等塑膠在本質上不容易分解。

【0004】 應對塑膠廢料產品問題之方法典型地包括焚化、填埋棄置及機械崩解。然而，此等方法亦具有顯著環境影響。舉例而言，塑膠之焚化產生釋放至大氣中之潛在有害的副產物；塑膠在填埋場中之分解速率通

常極緩慢且存在毒性材料將滲入地下水中之風險；而機械崩解相對昂貴且低效，且其副產物通常用途有限。

【0005】近年來，塑膠之生物學(酵素性)降解已被視為減少塑膠廢料累積之替代方法。此方法包括使用PET酶，其為一種催化PET水解成單體對苯二甲酸單-2-羥乙酯(MHET)之酵素的酯酶類別，且所得MHET藉由MHET酶之作用進一步分解成對苯二甲酸及乙二醇。對苯二甲酸為聚酯PET之前驅體。

【0006】儘管塑膠之酵素性降解為緩解塑膠廢料產品之環境影響及其棄置的引人注目之替代方案，但尚未發現廣泛採用，包括因為其在常見工業宿主生物體中之相對低效、緩慢酵素性降解速率及低酵素表現量。因此，仍迫切需要用於塑膠之酵素性降解的改良方法及試劑。

【發明內容】

【0007】在本文所揭示之一個態樣中，提供一種具有酯酶活性之多肽，其中該酯酶活性能夠將對苯二甲酸單酯轉化成對苯二甲酸及醇；將對苯二甲酸二酯轉化成對苯二甲酸單酯及醇；或將對苯二甲酸二酯轉化成對苯二甲酸及醇；其中該多肽包含選自由以下組成之群的胺基酸序列：SEQ ID NO:2之胺基酸5-261或與其具有至少85%序列一致性之胺基酸序列；SEQ ID NO: 3之胺基酸5-261或與其具有至少77%序列一致性之胺基酸序列；SEQ ID NO: 4之胺基酸5-261或與其具有至少75%序列一致性之胺基酸序列；SEQ ID NO: 5之胺基酸5-261或與其具有至少95%序列一致性之胺基酸序列；及SEQ ID NO: 6之胺基酸5-261或與其具有至少96%序列一致性之胺基酸序列，且其中該多肽不為SEQ ID NO: 1或SEQ ID NO: 12。

【0008】在一實施例中，該多肽包含SEQ ID NO: 6之胺基酸5-261

之胺基酸序列或與其具有至少96%序列一致性之胺基酸序列。

【0009】 在一實施例中，該多肽包含SEQ ID NO: 7之胺基酸5-261之胺基酸序列或與其具有至少97%一致性之胺基酸序列。

【0010】 在一實施例中，該多肽包含SEQ ID NO: 8之胺基酸5-261之胺基酸序列或與其具有至少96%一致性之胺基酸序列。

【0011】 在一實施例中，該多肽包含SEQ ID NO: 9之胺基酸5-261之胺基酸序列或與其具有至少97%一致性之胺基酸序列。

【0012】 在一實施例中，該多肽包含SEQ ID NO: 10之胺基酸5-261之胺基酸序列或與其具有至少98%一致性之胺基酸序列。

【0013】 在一實施例中，該多肽包含SEQ ID NO: 11之胺基酸5-261之胺基酸序列或與其具有至少98%一致性之胺基酸序列。

【0014】 在另一實施例中，該多肽包含SEQ ID NO: 119之胺基酸序列。

【0015】 在另一態樣中，提供一種具有酯酶活性之多肽，其中該酯酶活性能夠將對苯二甲酸單酯轉化成對苯二甲酸及醇；將對苯二甲酸二酯轉化成對苯二甲酸單酯及醇；或將對苯二甲酸二酯轉化成對苯二甲酸及醇，其中該多肽包含SEQ ID NO: 119之胺基酸序列。

【0016】 在本文所揭示之另一態樣中，提供一種用於酵素水解作為PET降解之副產物產生的對苯二甲酸單酯及/或對苯二甲酸二酯之方法，其中該對苯二甲酸單酯為視情況經苯甲基取代之對苯二甲酸單-C₁-C₁₀烷基酯，且其中該對苯二甲酸單酯不為對苯二甲酸單-(2-羥乙酯) (MHET)；該對苯二甲酸二酯為視情況經苯甲基取代之對苯二甲酸二-C₁-C₁₀烷基酯；該方法包含使該對苯二甲酸單酯及/或對苯二甲酸二酯在條件下暴露

於具有酯酶活性之多肽，該等條件足以使該多肽能夠：將該對苯二甲酸單酯轉化成對苯二甲酸及醇；將該對苯二甲酸二酯轉化成對苯二甲酸單酯及醇；或將該對苯二甲酸二酯轉化成對苯二甲酸及醇。

【0017】 在一實施例中，該多肽包含SEQ ID NO: 1之胺基酸序列或與SEQ ID NO: 1具有至少70%序列一致性之胺基酸序列。

【0018】 在一實施例中，該對苯二甲酸單酯係選自由以下組成之群：對苯二甲酸單苯甲酯(MBZT)、對苯二甲酸單己酯、對苯二甲酸單庚酯(MHPT)及對苯二甲酸單辛酯(MOCT)。在一較佳實施例中，對苯二甲酸單酯為MBZT或MOCT。在一個實施例中，該多肽包含SEQ ID NO: 9-11中任一者之胺基酸5-261之胺基酸序列或與前述任一者具有至少70%序列一致性之胺基酸序列。

【0019】 在一實施例中，該對苯二甲酸單酯係自由以下組成之群：對苯二甲酸二苯甲酯(DBZT)、對苯二甲酸二己酯(DHXT)、對苯二甲酸二庚酯(DHPT)及對苯二甲酸二辛酯(DOCT)。在一較佳實施例中，該對苯二甲酸單酯為DBZT或DOCT。在一個實施例中，該多肽包含SEQ ID NO: 6-8中任一者之胺基酸5-261之胺基酸序列或與前述任一者具有至少70%序列一致性之胺基酸序列。

【0020】 在一實施例中，該對苯二甲酸單酯及對苯二甲酸二酯係藉由水解或降解聚對苯二甲酸乙二酯(PET)來產生。

【0021】 本發明亦擴展至一種組合物，其包含如本文中所描述之多肽。

【0022】 本發明亦擴展至一種核酸序列，其編碼本文中所描述之多肽。

【0023】 本發明亦擴展至一種表現載體，其包含本文中所描述之核酸序列。

【0024】 本發明亦擴展至一種宿主細胞，其包含本文中所描述之核酸序列或表現載體。

【0025】 在另一態樣中，本發明提供一種產生具有酯酶活性之多肽之方法，該方法包含

- i) 提供本文中所描述之多核苷酸；
- ii) 在足以允許宿主細胞產生多肽之條件下在該宿主細胞中表現該多核苷酸；及
- iii) 收集藉由ii)中之該宿主細胞產生的該多肽。

【0026】 本發明亦擴展至一種降解包含聚酯之塑膠產品之方法，該方法包含在足以使多肽能夠降解塑膠產品之條件下使塑膠產品與本文中所描述之多肽、組合物或宿主細胞接觸。在一實施例中，塑膠產品包含聚酯聚對苯二甲酸乙二酯(PET)。

【0027】 本發明亦擴展至一種組合物，其包含藉由本文中所揭示之方法回收之對苯二甲酸及/或醇。

【0028】 在另一態樣中，提供一種宿主細胞，其經基因修飾以表現本文中所描述之多肽。

【0029】 在另一態樣中，提供一種使用藉由本文中所揭示之方法產生的對苯二甲酸及醇之組合物產生塑膠產品的方法。

【圖式簡單說明】

【0030】 圖1展示以下酵素性轉化之示意圖：(i)將對苯二甲酸單酯轉化成對苯二甲酸及醇；(ii)將對苯二甲酸二酯轉化成對苯二甲酸單酯及

醇；及(iii)將對苯二甲酸二酯轉化成對苯二甲酸及醇。在此示意圖中，X為C₁-C₁₀且重組C₁-C₁₀單醇由X-OH表示。

【0031】圖2展現本文中所揭示之具有SEQ ID NO 69-118 (自左至右)之胺基酸序列的多肽之DOCT酶活性，藉由單體等效水解產品MOCT及TPA (mg/mL)之組合濃度所測定，藉由U/HPLC所測定(如Y軸上所展現)。SEQ ID NO提供於X軸上。

【實施方式】

【0032】本申請案主張於2022年8月26日申請之名稱為「Enzymes and uses thereof」之澳大利亞臨時申請案第2022902457號之優先權，該申請案之內容以全文引用之方式併入本文中。

【0033】除非另外定義，否則本文所用之所有技術及科學術語具有與一般熟習本發明所屬技術者通常所瞭解相同的含義。雖然與本文所描述之方法及材料類似或等效的任何方法及材料可用於本發明之實踐或測試，但描述較佳方法及材料。出於本發明之目的，以下術語定義如下。

【0034】除非另外明確陳述，否則冠詞「一(a/an)」在本文中用於指一個或多於一個(亦即至少一個)文法冠詞對象。舉例而言，「一元件」意謂一個元件或大於一個元件。

【0035】如本文中所使用，術語「約」係指變化達參考數量、含量、值、尺寸、大小或量之至多10% (例如，達10%、9%、8%、7%、6%、5%、4%、3%、2%或1%)之數量、含量、值、尺寸、大小或量。

【0036】在整個本說明書中，除非上下文另有要求，否則字組「包含(comprise/comprises/comprising)」應理解為意味著包括所陳述步驟或要素或一組步驟或要素，但不排除任何其他步驟或要素或其他組步驟或要

素。

【0037】 雖然藉由PET酶酵素將PET酵素性轉化成單體對苯二甲酸單-2-羥乙酯(MHET)及雙-(2-羥乙基)對苯二甲酸(BHET)及藉由MHET酶將MHET酵素水解成對苯二甲酸酯/TPA為已知的(MHET酶最初與PET酶一起發現於細菌大阪堺菌(*Ideonella sakaiensis*)中；該兩種酵素使得細菌能夠以塑膠PET作為碳源而生存；Yoshida等人(2016) *Science* 351: 1196))，但能夠降解或水解其他塑膠或PET中間物，包括對苯二甲酸酯(特別係除MHET及BHET以外的對苯二甲酸二酯及單酯，參見例如Ion等人，2021 *Catalysis Today* 366:177)之酵素仍待識別。本發明至少部分基於本發明人提出之酯酶識別；該等酯酶對PET之對苯二甲酸單-酯及對苯二甲酸二-酯，諸如對苯二甲酸單及二-C₁-C₁₀烷基酯，特別係對苯二甲酸單及二-C₆-C₁₀烷基酯具有出人意料的水解酶/酯酶活性。

【0038】 因此，在本文所揭示之一個態樣中，提供一種具有酯酶活性之多肽，其中該酯酶活性能夠將對苯二甲酸單酯轉化成對苯二甲酸及醇；將對苯二甲酸二酯轉化成對苯二甲酸單酯及醇；或將對苯二甲酸二酯轉化成對苯二甲酸及醇；其中該多肽包含選自由以下組成之群的胺基酸序列：SEQ ID NO:2之胺基酸5-261或與其具有至少85%序列一致性之胺基酸序列；SEQ ID NO: 3之胺基酸5-261或與其具有至少77%序列一致性之胺基酸序列；SEQ ID NO: 4之胺基酸5-261或與其具有至少75%序列一致性之胺基酸序列；SEQ ID NO: 5之胺基酸5-261或與其具有至少95%序列一致性之胺基酸序列；及SEQ ID NO: 6之胺基酸5-261或與其具有至少96%序列一致性之胺基酸序列，其中該多肽不為SEQ ID NO: 1或SEQ ID NO: 12。

【0039】 在一實施例中，對苯二甲酸單酯為視情況經苯甲基取代之對苯二甲酸單-C1-C10烷基酯，且對苯二甲酸二酯為視情況經苯甲基取代之對苯二甲酸二-C1-C10烷基酯。在一較佳實施例中，對苯二甲酸單酯係選自由以下組成之群：對苯二甲酸單苯甲酯(MBZT)、對苯二甲酸單己酯(MHXT)、對苯二甲酸單庚酯(MHPT)及對苯二甲酸單辛酯(MOCT)，對苯二甲酸二酯係選自由以下組成之群：對苯二甲酸二苯甲酯(DBZT)、對苯二甲酸二己酯(DHXT)、對苯二甲酸二庚酯(DHPT)及對苯二甲酸二辛酯(DOCT)。

【0040】 如本文中所使用，對於對苯二甲酸之指代亦擴展至對苯二甲酸之鹽。因此，在一實施例中，對苯二甲酸為對苯二甲酸之鹽。在一實施例中，對苯二甲酸之鹽為金屬鹼金屬鹽。在另一實施例中，所產生之對苯二甲酸為對苯二甲酸二鈉鹽。

【0041】 「至少85%」意謂多肽與SEQ ID NO: 2或與SEQ ID NO: 2之胺基酸5-261共用至少85%、較佳地至少87%、較佳地至少88%、較佳地至少90%、較佳地至少92%、較佳地至少94%、較佳地至少95%、較佳地至少96%、較佳地至少97%、較佳地至少98%或更佳地至少99%序列一致性。應理解，由於本文中所描述之多肽為合成多肽，因此在此上下文中，「至少85%」可包括跨SEQ ID NO: 2之完整序列或跨SEQ ID NO: 2之胺基酸5-261的完整胺基酸序列之100%序列一致性。

【0042】 「至少77%」意謂多肽與SEQ ID NO: 3或與SEQ ID NO: 3之胺基酸5-261共用至少77%、較佳地至少80%、較佳地至少85%、較佳地至少90%、較佳地至少92%、較佳地至少94%、較佳地至少95%、較佳地至少96%、較佳地至少97%、較佳地至少98%或更佳地至少99%序列一

致性。應理解，由於本文中所描述之多肽為合成多肽，因此在此上下文中，「至少77%」可包括跨SEQ ID NO: 3之完整序列或跨SEQ ID NO: 3之胺基酸5-261的完整序列之100%序列一致性。

【0043】 「至少75%」意謂多肽與SEQ ID NO: 4或與SEQ ID NO: 4之胺基酸5-261共用至少75%、較佳地至少80%、較佳地至少85%、較佳地至少90%、較佳地至少92%、較佳地至少94%、較佳地至少95%、較佳地至少96%、較佳地至少97%、較佳地至少98%或更佳地至少99%序列一致性。應理解，由於本文中所描述之多肽為合成多肽，因此在此上下文中，「至少85%」可包括跨SEQ ID NO: 4之完整序列或跨SEQ ID NO: 4之胺基酸5-261的完整序列之100%序列一致性。

【0044】 「至少95%」意謂多肽與SEQ ID NO: 5或與SEQ ID NO: 5之胺基酸5-261共用至少95%、較佳地至少96%、較佳地至少97%、較佳地至少98%或更佳地至少99%序列一致性。應理解，由於本文中所描述之多肽為合成多肽，因此在此上下文中，「至少95%」可包括跨SEQ ID NO: 5之完整序列或跨SEQ ID NO: 5之胺基酸5-261之100%序列一致性。

【0045】 「至少96%」意謂多肽與SEQ ID NO: 6或與SEQ ID NO: 6之胺基酸5-261共用至少96%、較佳地至少97%、較佳地至少98%或更佳地至少99%序列一致性。應理解，由於本文中所描述之多肽為合成多肽，因此在此上下文中，「至少95%」可包括跨SEQ ID NO: 6之完整序列或跨SEQ ID NO: 6之胺基酸5-261的完整序列之100%序列一致性。

【0046】 在一實施例中，多肽包含SEQ ID NO: 2之胺基酸5-261之胺基酸序列或與其具有至少85%序列一致性之胺基酸序列。在另一實施例中，多肽由SEQ ID NO: 2之胺基酸序列組成。

【0047】 在一實施例中，多肽包含SEQ ID NO: 3之胺基酸5-261之胺基酸序列或與其具有至少77%序列一致性之胺基酸序列。在另一實施例中，多肽由SEQ ID NO: 3之胺基酸序列組成。

【0048】 在一實施例中，多肽包含SEQ ID NO: 4之胺基酸5-261之胺基酸序列或與其具有至少75%序列一致性之胺基酸序列。在另一實施例中，多肽由SEQ ID NO: 4之胺基酸序列組成。

【0049】 在一實施例中，多肽包含SEQ ID NO: 5之胺基酸5-261之胺基酸序列或與其具有至少95%序列一致性之胺基酸序列。在另一實施例中，多肽由SEQ ID NO: 5之胺基酸序列組成。

【0050】 在一實施例中，多肽包含SEQ ID NO: 6之胺基酸5-261之胺基酸序列或與其具有至少96%序列一致性之胺基酸序列。

【0051】 在另一實施例中，多肽由SEQ ID NO: 6之胺基酸序列組成。

【0052】 在一個實施例中，多肽包含SEQ ID NO: 7之胺基酸5-261之胺基酸序列。在一個實施例中，多肽包含SEQ ID NO: 8之胺基酸5-261之胺基酸序列。在一個實施例中，多肽包含SEQ ID NO: 9之胺基酸5-261之胺基酸序列。在一個實施例中，多肽包含SEQ ID NO: 10之胺基酸5-261之胺基酸序列。在一個實施例中，多肽包含SEQ ID NO: 11之胺基酸5-261之胺基酸序列。

【0053】 在另一實施例中，多肽由SEQ ID NO: 6之胺基酸序列組成或基本上由其組成。在一個實施例中，多肽由SEQ ID NO: 7之胺基酸序列組成或基本上由其組成。在一個實施例中，多肽由SEQ ID NO: 8組成或基本上由其組成。在一個實施例中，多肽由SEQ ID NO: 9組成或基本上由其

組成。在一個實施例中，多肽由SEQ ID NO: 10組成或基本上由其組成。

在一個實施例中，多肽由SEQ ID NO: 11組成或基本上由其組成。

【0054】 在一實施例中，多肽包含SEQ ID NO: 1-118中任一者之胺基酸5-260之胺基酸序列。

【0055】 在一實施例中，多肽包含SEQ ID NO: 119之胺基酸序列。
在一實施例中，SEQ ID NO: 119之位置X處之胺基酸殘基係選自SEQ ID NO: 1-118中任一者之對應位置處之胺基酸殘基。

【0056】 在另一實施例中，多肽進一步包含N端細胞輸出信號肽或選自SEQ ID NO: 120 (MAAN)；SEQ ID NO: 121 (MAEN)；SEQ ID NO: 122 (MQAN)；SEQ ID NO: 123 (MADN)；及SEQ ID NO: 124 (MQSN)之胺基酸序列之信號肽。

【0057】 對苯二甲酸單酯對熟習此項技術者而言將為熟悉的。舉例而言，如本文所使用，術語對苯二甲酸單酯係指1,4二取代之苯，其中取代為羧酸官能基及酯官能基。對苯二甲酸單酯包括對苯二甲酸單-烷基酯。在一些實施例中，對苯二甲酸單酯係經由用C₁-C₁₀單醇使PET轉酯化來形成。在特定實施例中，對苯二甲酸單酯係用C₆-C₁₀單醇使PET轉酯化來形成。在特定實施例中，對苯二甲酸單酯係經由用苯甲醇、己醇、庚醇或辛醇使PET轉酯化來形成。

【0058】 對苯二甲酸二酯對熟習此項技術者而言將為熟悉的。舉例而言，如本文所使用，術語對苯二甲酸二酯係指1,4二取代之苯，其中取代為酯官能基。對苯二甲酸二酯包括對苯二甲酸二-烷基酯。在一些實施例中，對苯二甲酸二酯係經由用C₁-C₁₀單醇使PET轉酯化來形成。在特定實施例中，對苯二甲酸二酯係用C₆-C₁₀單醇使PET轉酯化來形成。在特定

實施例中，對苯二甲酸二酯係經由用苯甲醇、己醇、庚醇或辛醇使PET轉酯化來形成。

【0059】 本發明亦擴展至一種組合物，其包含如本文中所描述之多肽。

【0060】 本發明亦擴展至一種核酸序列，其編碼本文中所描述之多肽。

【0061】 本發明亦擴展至一種表現載體，其包含本文中所描述之核酸序列。

【0062】 本發明亦擴展至一種宿主細胞，其包含本文中所描述之核酸序列或表現載體。

【0063】 在另一態樣中，本發明提供一種產生具有酯酶活性之多肽之方法，該方法包含

- i) 提供本文中所描述之多核苷酸；
- ii) 在足以允許宿主細胞產生多肽之條件下在該宿主細胞中表現該多核苷酸；及
- iii) 收集藉由ii)中之該宿主細胞產生的該多肽。

【0064】 在又一態樣中，提供一種用於酵素水解作為PET降解之副產物產生的對苯二甲酸單酯及/或對苯二甲酸二酯之方法，其中

- a. 對苯二甲酸單酯為視情況經苯甲基取代之對苯二甲酸單- C_1 - C_{10} 烷基酯，且其中該對苯二甲酸單酯不為對苯二甲酸單-(2-羥乙酯) (MHET)；
- b. 該對苯二甲酸二酯為視情況經苯甲基取代之對苯二甲酸二- C_1 - C_{10} 烷基酯；

該方法包含使該對苯二甲酸單酯及/或對苯二甲酸二酯在條件下暴露

於具有酯酶活性之多肽，該等條件足以使該多肽能夠：

- i. 將該對苯二甲酸單酯轉化成對苯二甲酸及醇；
- ii. 將該對苯二甲酸二酯轉化成對苯二甲酸單酯及醇；或
- iii. 將該對苯二甲酸二酯轉化成對苯二甲酸及醇。

【0065】 在一個實施例上，用於水解作為PET降解之副產物產生的對苯二甲酸單酯及/或對苯二甲酸二酯之多肽包含SEQ ID NO: 1之胺基酸序列或與SEQ ID NO: 1具有至少70%序列一致性之胺基酸序列。

【0066】 在一實施例中，對苯二甲酸單酯為視情況經苯甲基取代之對苯二甲酸單-C₆-C₁₀烷基酯。在一個實施例中，酯為單-C₆烷基酯。在一個實施例中，酯為單-C₇烷基酯。在一個實施例中，酯為單-C₈烷基酯。在另一實施例中，酯為單-C₉烷基酯。在另一實施例中，酯為單-C₁₀烷基酯。在一實施例中，該對苯二甲酸單酯係選自由以下組成之群：對苯二甲酸單苯甲酯(MBZT)、對苯二甲酸單己酯、對苯二甲酸單庚酯(MHPT)及對苯二甲酸單辛酯(MOCT)。在一較佳實施例中，對苯二甲酸單酯為MBZT或MOCT。在一較佳實施例中，多肽包含SEQ ID NO: 9-11中任一者之胺基酸序列及與前述任一者具有至少70%序列一致性之胺基酸序列。

【0067】 在一實施例中，對苯二甲酸單酯為視情況經苯甲基取代之對苯二甲酸二-C₆-C₁₀烷基酯。在一個實施例中，酯為二-C₆烷基酯。在一個實施例中，酯為二-C₇烷基酯。在一個實施例中，酯為二-C₈烷基酯。在另一實施例中，酯為二-C₉烷基酯。在另一實施例中，酯為二-C₁₀烷基酯。在一實施例中，該對苯二甲酸二酯係自由以下組成之群：對苯二甲酸二苯甲酯(DBZT)、對苯二甲酸二己酯(DHXT)、對苯二甲酸二庚酯(DHPT)及對苯二甲酸二辛酯(DOCT)。在一較佳實施例中，該對苯二甲酸

單酯為DBZT或DOCT。在一較佳實施例中，多肽包含SEQ ID NO: 6-8中任一者之胺基酸序列及與前述任一者具有至少70%序列一致性之胺基酸序列。

【0068】 在一實施例中，該對苯二甲酸單酯及對苯二甲酸二酯係藉由水解或降解聚對苯二甲酸乙二酯(PET)來產生。在另一實施例中，對苯二甲酸單酯及對苯二甲酸二酯係藉由包含以下之製程來產生：使PET暴露於氫氧化鈉，及/或使PET與酯酶接觸。在一較佳實施例中，對苯二甲酸單酯及對苯二甲酸二酯係藉由包含以下之製程來產生：使PET經歷利用C₆-C₁₀單醇之鹼催化之轉酯化；及/或使該PET與酯酶接觸。在一較佳實施例中，C₆-C₁₀單醇為苯甲醇、辛醇或庚醇。在又另一較佳實施例中，C₆-C₁₀單醇為1-辛醇。

【0069】 本發明亦擴展至一種降解包含聚酯之塑膠產品之方法，該方法包含在足以使多肽能夠降解塑膠產品之條件下使塑膠產品與本文中所描述之多肽、組合物或宿主細胞接觸。

【0070】 在一個實施例中，聚酯係選自由以下組成之群：聚乳酸(PLA)、聚對苯二甲酸丙二酯(PTT)、聚對苯二甲酸丁二酯(PBT)、聚異山梨醇對苯二甲酸乙二酯(PEIT)、聚對苯二甲酸乙二酯(PET)、聚羥基烷酸酯(PHA)、聚丁二酸丁二酯(PBS)、聚丁二酸己二酸丁二酯(PBSA)、聚己二酸對苯二甲酸丁二酯(PBAT)、聚呋喃酸乙二酯(PEF)、聚己內酯(PCL)、聚己二酸乙二酯(PEA)、聚(乙醇酸)(PGA)、聚(乳酸-共-乙醇酸)(PLGA)及前述任一者之組合。在一實施例中，聚酯為聚對苯二甲酸乙二酯(PET)。

【0071】 本發明亦擴展至一種組合物，其包含藉由本文中所揭示之

方法回收之對苯二甲酸及/或乙二醇。

【0072】 在另一態樣中，提供一種宿主細胞，其經基因修飾以表現本文中所描述之多肽。

【0073】 在另一態樣中，提供一種使用藉由本文所揭示之方法產生之對苯二甲酸及乙二醇之組合物產生塑膠產品的方法。

【0074】 術語「野生型」在本文中用於表示多肽之天然存在之同功異型物；亦即，如在自然界中呈現。術語「現存」用於表示仍存在(亦即，未滅絕)之生物體或物種中發現之多肽的天然存在之同共異形物。

【0075】 現存角質酶之實例對熟習此項技術者而言將為熟悉的。現存角質酶之實例包括但不限於：葉枝堆肥角質酶(Leaf-branch compost cutinase) (LCC；登錄號：G9BY57)、TfCut2 (源於嗜熱放線菌(*Thermobifida fusca*)之角質酶；登錄號：E5BBQ3)、E9LVH9 (源於嗜熱纖維菌(*Thermobifida cellulosilytica*)之Cut2)及E5BBQ2 (源於嗜熱放線菌之Cut.1-KW3角質酶)。

【0076】 在本文中，術語「肽」、「多肽」、「蛋白質」、「酵素」應理解為指代藉由肽鍵連接之胺基酸鏈，與形成該鏈之胺基酸數目無關。胺基酸通常根據以下命名法由其一個字母或三個字母碼表示：A：丙胺酸(Ala)；C：半胱胺酸(Cys)；D：天冬胺酸(Asp)；E：麩胺酸(Glu)；F：苯丙胺酸(Phe)；G：甘胺酸(Gly)；H：組胺酸(His)；I：異白胺酸(Ile)；K：離胺酸(Lys)；L：白胺酸(Leu)；M：甲硫胺酸(Met)；N：天冬醯胺(Asn)；P：脯胺酸(Pro)；Q：麩醯胺酸(Gln)；R：精胺酸(Arg)；S：絲胺酸(Ser)；T：蘇胺酸(Thr)；V：纈胺酸(Val)；W：色胺酸(Trp)及Y：酪胺酸(Tyr)。

【0077】如本文中所使用之術語「水解酶」通常係指屬於根據酵素命名法(Enzyme Nomenclature)分類為EC 3的一類水解酶的酵素，其催化化學鍵(包括酯鍵)水解。

【0078】如本文中所使用之術語「酯酶」通常係指根據酵素命名法分類為EC 3.1的水解酶酵素，其催化酯鍵水解以產生酸及醇。術語「角質酶」係指根據酵素命名法分類為EC 3.1.1.74之絲胺酸酯酶酵素，其催化角質聚合物(由羥基及羥基環氧脂肪酸構成之聚酯)水解成角質單體。

【0079】如本文中所使用之術語「PET酶」通常係指根據酵素命名法分類為EC 3.1.1.101之酯酶酵素，其催化聚對苯二甲酸乙二酯(PET)塑膠水解成單體對苯二甲酸單-2-羥乙酯(MHET)。

【0080】在本文中，本發明之多肽應理解為具有水解酶或酯酶活性，能夠催化對苯二甲酸單-酯及對苯二甲酸二-酯水解。

【0081】如Palm等人指出(2019, *Nat. Comms.* 10:1717)，最近發現之特異性降解聚對苯二甲酸乙二酯(PET)之兩種細菌酵素表示對以其他方式造成環境負擔的含聚酯產品之有前景的解決方案。首先，結構上充分表徵 α/β -水解酶摺疊酵素之大阪堺菌PET酶將PET轉化成對苯二甲酸單-(2-羥乙酯)(MHET)。作為第二關鍵酵素之MHET酶將MHET水解成對苯二甲酸酯及乙二醇(Palm等人(2019, *Nat. Comm.*, 10:1717)，Sagong等人(2020, *ACS Catal.* 10:4805)及Yoshida等人(2016, *Science*, 352(6278):1196))。

【0082】術語「突變體」及「變體」可在本文中互換使用以指包含衍生自SEQ ID NO: 1之胺基酸序列且進一步包含於一或多個(例如若干)位置修飾或改變(例如取代、插入及/或缺失)之多肽，且當與現存PET酶或具

有PET酶活性之角質酶或SEQ ID NO: 1之多肽相比時在催化對苯二甲酸單酯及對苯二甲酸二酯水解中具有增強之酯酶活性。此類變體可藉由熟此項技術中熟知之多種技術獲得，其說明性實例包括定點誘變、無規誘變及合成寡核苷酸構築。如本文中關於胺基酸殘基或位置所使用之術語「修飾」、「改變」、「取代」以及類似者通常意謂與野生型或親本多肽之胺基酸相比，特定位置中之胺基酸已經修飾。

【0083】 適合之取代可包括一胺基酸殘基藉由選自以下之另一胺基酸殘基替代：天然存在之20種標準胺基酸殘基、天然存在之稀有胺基酸殘基(例如羥基脯胺酸、羥基離胺酸、別(allo)羥基離胺酸、6-N-甲基離胺酸、N-乙基甘胺酸、N-甲基甘胺酸、N-乙基天冬醯胺酸、別異白胺酸、N-甲基異白胺酸、N-甲基纈胺酸、焦麩醯胺酸、胺基丁酸、鳥胺酸、正白胺酸、正纈胺酸)及通常以合成方式製得的非天然存在之胺基酸殘基(例如環己基-丙胺酸)。較佳地，取代包含一胺基酸殘基藉由選自以下之另一胺基酸殘基替代：20種天然存在之標準胺基酸殘基(G、P、A、V、L、I、M、C、F、Y、W、H、K、R、Q、N、E、D、S及T)。修飾或改變可在本文中使用以下術語識別：Y197V表示親本多肽序列之位置197之胺基酸殘基酪胺酸(Y)經纈胺酸(V)取代。Y197V/I/M表示親本序列之位置197之胺基酸殘基酪胺酸(Y)可經以下胺基酸中之一者取代：纈胺酸(V)、異白胺酸(I)或甲硫胺酸(M)。取代可為保守性或非保守性取代。保守性取代之實例將為熟習此項技術者所熟悉的，其說明性實例包括鹼性胺基酸(精胺酸、離胺酸及組胺酸)、酸性胺基酸(麩胺酸及天冬胺酸)、極性胺基酸(麩醯胺酸、天冬醯胺酸及蘇胺酸)、疏水性胺基酸(甲硫胺酸、白胺酸、異白胺酸、半胱胺酸及纈胺酸)、芳族胺基酸(苯丙胺酸、色胺酸及酪胺酸)及

小胺基酸(甘胺酸、丙胺酸及絲胺酸)之群內的取代。

【0084】 本申請案中所揭示之位置係參考所述SEQ ID NOs中所闡述之胺基酸序列編號。在此方面，術語「對應於」，當關於胺基酸位置使用時，欲意謂當該位置與參考序列中所述序列中之相等或對應位置比對時，在一多肽序列中之胺基酸位置。舉例而言，SEQ ID NO: 6之位置5之胺基酸殘基(胺基酸P/脯胺酸)將對應於SEQ ID NO: 116之SEQ ID NO: 4之位置4之胺基酸殘基(胺基酸P/脯胺酸)。在另一實例中，SEQ ID NO: 19之位置14之胺基酸殘基(胺基酸N/天冬醯胺酸)將對應於SEQ ID NO: 113之位置13之胺基酸殘基(胺基酸E/麩胺酸)。在另一實例中，在SEQ ID NO: 119中，SEQ ID NO: 119之位置4的胺基酸殘基(胺基酸P/脯胺酸)將對應於SEQ ID NO: 44之位置12的胺基酸殘基(胺基酸P/脯胺酸)；且SEQ ID NO: 119之位置3的胺基酸殘基將對應於SEQ ID NO: 44之位置11的胺基酸殘基(胺基酸A/丙胺酸)。在另一實例中，在SEQ ID NO: 119中，SEQ ID NO: 119之位置3的胺基酸殘基將對應於SEQ ID NO: 105之位置10的胺基酸殘基(胺基酸D/天冬胺酸)。

【0085】 如本文中所使用，術語「序列一致性(sequence identity)」或「一致性(identity)」係指兩個多肽序列之間的匹配(一致胺基酸殘基)之數目(或表示為百分比%之分數)。在一較佳實施例中，序列一致性係藉由在比對以使重疊及一致性達到最大，同時使序列間隙減至最小時比較序列來判定。視兩個序列之長度而定，序列一致性可使用熟習此項技術者已知之多種數學全局或局部比對演算法中之任一者來判定。類似長度之序列可使用最佳地在整個長度上比對序列之全局比對演算法(例如，尼德曼-翁施演算法(Needleman and Wunsch algorithm)；Needleman及Wunsch，

1970)比對，而大體上不同長度之序列較佳使用局部比對演算法(例如，史密斯-沃特曼演算法 (Smith and Waterman algorithm) (Smith 及 Waterman, 1981)或阿爾丘爾演算法(Altschul algorithm) (Altschul等人, 1997; Altschul等人, 2005))比對。出於判定胺基酸序列一致性百分比之目的之比對可藉由熟習此項技術者可用之任何方式達成，其說明性實例包括公開可用之電腦軟體，諸如可在 <http://blast.ncbi.nlm.nih.gov/> 或 <http://www.ebi.ac.uk/Tools/emboss/> 獲得。熟習此項技術者可易於判定用於量測比對之適當參數，包括在所比較序列之全長上達成最大比對所需之任何演算法。如本文中所使用，序列一致性%通常係指使用創建兩個序列之理想全局比對(例如使用尼德曼-翁施演算法)之雙序列比對產生的值，其中全部搜尋參數經設定成既定值，例如，計分矩陣 = BLOSUM62，開頭間隙 = 10，延伸間隙 = 0.5，端間隙罰分 = 假，端開頭間隙 = 10及端延伸間隙 = 0.5。

【0086】 如本文中所使用，術語「重組」通常係指藉由基因工程化產生之核酸構築體、載體、多肽或細胞。

【0087】 如本文中所使用，術語「表現」通常係指諸如藉由轉錄、轉錄後修飾、轉譯、轉譯後修飾及分泌而參與多肽產生之任何步驟。

【0088】 術語「表現卡匣」表示包含編碼區及適當地可操作地連接編碼區之調節區的核酸構築體。

【0089】 術語「表現載體」通常意謂包含表現卡匣之DNA或RNA分子。表現載體可為線性或環狀雙股DNA分子。

【0090】 如本文中所使用，術語「聚合物」通常係指其結構由共價化學鍵連接之多個單體(重複單元)構成之化合物或化合物之混合物。在本

發明之上下文內，術語聚合物包括天然或合成聚合物，其由單一類型之重複單元(亦即，均聚物)或由不同重複單元之混合物(亦即，共聚物或雜聚物)構成。

【0091】 如本文中所使用，術語「含聚酯材料」、「含聚酯產品」及其類似者應理解為係指包含至少一種呈結晶、半結晶或完全非晶形式之聚酯的產品，諸如塑膠產品。含聚酯材料可指由含有至少一種聚酯且可能其他物質或諸如塑化劑、礦物質或有機填充劑之添加劑的至少一種塑膠材料製成的任何物品，該至少一種塑膠材料諸如塑膠片材、管、棒、型材、成型件、薄膜、大塊體、纖維、紡織物等。在一實施例中，含聚酯材料為包含至少一種含聚酯纖維之織物或織品。在另一實施例中，含聚酯材料為適用於製造塑膠產品之呈熔融或固體狀態的塑膠化合物或塑膠調配物。

【0092】 適合之聚酯將為熟習此項技術者熟悉的，其說明性實例包括聚乳酸(PLA)、聚對苯二甲酸乙二酯(PET)、聚對苯二甲酸丙二酯(PTT)、聚對苯二甲酸丁二酯(PBT)、聚異山梨醇對苯二甲酸乙二酯(PEIT)、聚羥基烷酸酯(PHA)、聚丁二酸丁二酯(PBS)、聚丁二酸己二酸丁二酯(PBSA)、聚己二酸對苯二甲酸丁二酯(PBAT)、聚呋喃酸乙二酯(PEF)、聚己內酯(PCL)及聚(己二酸乙二酯)(PEA)。因此，在一實施例中，聚酯係選自由以下組成之群：聚乳酸(PLA)、聚對苯二甲酸乙二酯(PET)、聚對苯二甲酸丙二酯(PTT)、聚對苯二甲酸丁二酯(PBT)、聚異山梨醇對苯二甲酸乙二酯(PEIT)、聚羥基烷酸酯(PHA)、聚丁二酸丁二酯(PBS)、聚丁二酸己二酸丁二酯(PBSA)、聚己二酸對苯二甲酸丁二酯(PBAT)、聚呋喃酸乙二酯(PEF)、聚己內酯(PCL)、聚(己二酸乙二酯)(PEA)及前述任一者之組合。

【0093】 測定或量測多肽之酯酶/水解酶活性之適合方法將為熟習此項技術者所熟悉的，其說明性實例描述於本文別處。其他說明性實例描述於Palm等人(2019, *Nat. Comm.*, 10:1717)，Sagong等人(2020, *ACS Catal.* 10:4805)及Yoshida等人(2016, *Science*, 352(6278):1196)中，該等參考之內容以全文引用之方式併入本文中。適用於測定或量測多肽之酯酶/水解酶活性之另一方法係藉由量測使用分析型高效液相層析(HPLC)產生之對苯二甲酸或對苯二甲酸單酯的量。

【0094】 新穎工程化多肽及具有酯酶活性之變體的酯酶活性可指定為相對於比較劑之酯酶活性的絕對值或值。在一實施例中，酯酶活性量測為在適合溫度、pH及緩衝液條件下每小時及每毫克或莫耳酵素釋放之單體及/或寡聚物(例如以毫克或莫耳計)的速率。在本發明之一實施例中，每小時釋放之單體及/或寡聚物之速率在約1 mol/h/mol酵素至約500 mol/h/mol酵素範圍內。在另一實施例中，每小時釋放之單體及/或寡聚物(例如以毫克計)之速率在約50 mol/h/mol酵素至約400 mol/h/mol酵素範圍內。在又一實施例中，每小時釋放之單體及/或寡聚物(例如以毫克計)之速率在約100 mol/h/mol酵素至約350 mol/h/mol酵素範圍內。

【0095】 有利地，本文中所描述之多肽可能夠至少在約10°C至約80°C、約20°C至約80°C、約30°C至約80°C、約30°C至約70°C、較佳地約40°C至約70°C、較佳地約50°C至約70°C或甚至更佳地約50°C至約60°C的溫度範圍內催化對苯二甲酸單-酯及對苯二甲酸二-酯水解。在一實施例中，本文中所描述之多肽在約10°C至約80°C，約20°C至約80°C，約30°C至約80°C，約30°C至約70°C，較佳地約40°C至約70°C，較佳地約50°C至約70°C或甚至更佳地約50°C至約60°C的溫度下展現酯酶活性。在一實施

例中，酯酶活性可在約10℃至約70℃，約20℃至約80℃，約30℃至約80℃，約30℃至約70℃，較佳地約40℃至約70℃，較佳地約50℃至約70℃或甚至更佳地約50℃至約60℃的溫度下量測。

【0096】 在一實施例中，具有酯酶活性之多肽包含水解酶活性或在約10℃至約80℃、約20℃至約80℃、約30℃至約80℃、約30℃至約70℃、較佳地約40℃至約70℃、較佳地約50℃至約70℃或甚至更佳地約50℃至約60℃的溫度下催化對苯二甲酸單-酯水解。

【0097】 在另一特定實施例中，具有酯酶活性之多肽將在約10℃至約80℃、約20℃至約80℃、約30℃至約80℃、約30℃至約70℃、較佳地約40℃至約70℃、較佳地約50℃至約70℃或甚至更佳地約50℃至約60℃的溫度下催化對苯二甲酸二-酯水解。

【0098】 在一實施例中，本文中所描述之多肽展現至少在約5至約11的pH範圍內，較佳地在約6至約10的pH範圍內，較佳地在約7至約10的pH範圍內，更佳地在約7.5至約9.5的pH範圍內的可量測水解酶/酯酶活性。

【0099】 在一實施例中，本文中所描述之多肽在催化對苯二甲酸單-酯水解中展現至少在約5至約11的pH範圍內，較佳地在約6至約10的pH範圍內，較佳地在約7至約10的pH範圍內，更佳地在約7.5至約9.5的pH範圍內之可量測水解酶/酯酶活性。

【0100】 在一實施例中，本文中所描述之多肽在催化對苯二甲酸二-酯水解中展現至少在約5至約11的pH範圍內，較佳地在約6至約10的pH範圍內，較佳地在約7至約10的pH範圍內，更佳地在約7.5至約9.5的pH範圍內之可量測水解酶/酯酶活性。

【0101】 在一實施例中，本文中所描述之多肽展現約40℃至約90℃、50℃至約90℃、較佳地約50℃至約80℃的熔融溫度(T_m)。

【0102】 如本文中所使用，術語「核酸」、「核酸序列」、「多核苷酸」、「寡核苷酸」及「核苷酸序列」可互換使用且係指一系列去氧核糖核苷酸及/或核糖核苷酸。核酸可為DNA (cDNA或gDNA)、RNA或兩者之混合物。該等核酸可呈單股形式或呈雙螺旋形式或兩者之混合物。該等核酸可具有重組、人工及/或合成來源，且可包含經修飾之核苷酸，包含例如經修飾之鍵、經修飾之嘌呤或嘧啶鹼基或經修飾之糖。本發明之核酸可呈經分離或純化形式，且藉由此項技術中本身已知的技術來製得、分離及/或操縱，該等技術例如cDNA文庫之選殖及表現、擴增、酵素性合成或重組技術。核酸亦可藉由熟知化學合成技術在活體外合成，如例如Belousov (1997) *Nucleic Acids Res.* 25:3440-3444中所描述。

【0103】 本文所揭示之核酸序列可適當地經密碼子最佳化。適用於密碼子最佳化之方法將為熟習此項技術者熟悉的，其說明性實例描述於參考手冊Sambrook等人(Sambrook等人，2001)中。

【0104】 本文中所描述之核酸序列可自本文中所描述之多肽之胺基酸序列推論，且密碼子使用可根據應轉錄核酸之宿主細胞來調適。

【0105】 在一些實施例中，本文中所描述之核酸序列可適當地包含其他核苷酸序列，諸如調節區，亦即啟動子、強化子、沉默子、終止子、信號肽及可用於引起或調節多肽在所選擇宿主細胞或系統中之表現的類似者。替代地或另外，本文中所描述之核酸序列可進一步包含編碼融合蛋白，諸如麥芽糖結合蛋白(MBP)或麩胱甘肽S轉移酶(GST)之其他核苷酸序列，其可用於促進多肽表現及/或溶解度。

【0106】 如本文中其他地方所指出，本發明亦擴展至包含本文中所描述之核酸序列的表現載體及表現卡匣，其視情況可操作地連接於引導核酸序列在適合宿主細胞中之表現的一或多個控制序列。典型地，表現載體或卡匣包含可操作地連接於控制序列，諸如轉錄啟動子及/或轉錄終止子之本文中所描述之核酸序列。控制序列可包括由宿主細胞或用於表現編碼本文中所描述之多肽之核酸的活體外表現系統識別之啟動子。啟動子將通常包含調節多肽之表現的轉錄控制序列。啟動子可為在宿主細胞中展示轉錄活性之任何多核苷酸，包括突變、截短及雜合啟動子，且可適合地自編碼細胞外或細胞內多肽之基因獲得，該等多肽與宿主細胞同源或異源。控制序列亦可為轉錄終止子，其由宿主細胞識別以終止轉錄。終止子通常可操作地連接於編碼多肽之核酸的3'端。在宿主細胞中具有功能之任何終止子可用於此上下文中。典型地，表現載體或卡匣包含可操作地連接於轉錄啟動子及轉錄終止子的本文中所描述之核酸序列。

【0107】 術語「載體」通常係指用作將重組遺傳物質轉移至宿主細胞中之媒介的DNA分子。適合之載體包括質體、噬菌體、病毒、F黏粒(fosmid)、黏質體及人工染色體。載體通常為包含插入序列(異源核酸序列，轉基因)及充當載體之「主鏈」之較大序列的DNA序列。將遺傳資訊轉移至宿主的載體之目的通常為在目標細胞中分離、倍增或表現插入序列。表現載體(亦稱為表現構築體)經特定調適以用於在目標細胞中表現異源序列，且通常具有驅動編碼多肽之異源序列之表現的啟動子序列。

【0108】 一般而言，用於表現載體中之調節元件包括轉錄啟動子、核糖體結合位點、終止子及視情況存在之操縱子。表現載體可進一步包含用於在宿主細胞中自主複製之複製起點、可選標記物、有限數目之適用限

制酵素位點及高複本數之可能性。適合之表現載體將為熟習此項技術者所熟悉的，其說明性實例包括選殖載體、經修飾之選殖載體、質體及病毒。能夠在不同宿主中提供適合之多肽表現量的表現載體亦為此項技術中所熟知的。載體之選擇將通常視載體與待引入載體之宿主細胞的相容性而定。

【0109】 本發明亦擴展至一種宿主細胞，其包含本文中所描述之核酸序列。宿主細胞可以瞬態或穩定方式轉型、轉染或轉導。核酸、表現卡匣或載體經引入至宿主細胞中，使得核酸、卡匣或載體作為染色體組成部分或作為自我複製的染色體外載體維持。術語「宿主細胞」涵蓋親本宿主細胞的歸因於複製期間發生之突變而與親本宿主細胞不一致之任何後代。宿主細胞可為適用於產生本發明之變體的任何細胞，例如原核細胞或真核細胞。原核宿主細胞可為任何革蘭氏陽性(Gram-positive)或革蘭氏陰性(Gram-negative)細菌。宿主細胞亦可為真核細胞，諸如酵母菌、真菌、哺乳動物、昆蟲或植物細胞。在一特定實施例中，宿主細胞係選自以下之群：大腸桿菌(*Escherichia coli*)、假單胞菌(*Pseudomonas*)、芽孢桿菌(*Bacillus*)、鏈黴菌(*Streptomyces*)、木黴菌(*Trichoderma*)、曲黴菌(*Aspergillus*)、酵母菌(*Saccharomyces*)、畢赤酵母菌(*Pichia*)、棲熱菌(*Thermus*)或耶氏酵母菌(*Yarrowia*)。

【0110】 根據本發明之核酸、表現卡匣或表現載體可藉由熟習此項技術者已知的任何方法而引入至宿主細胞中，該方法之說明性實例包括電穿孔、結合、轉導、勝任細胞轉化、原生質體轉化、原生質體融合、生物「基因槍(gene gun)」轉化、PEG介導之轉化、脂質輔助之轉化或轉染、化學方式介導之轉染、乙酸鋰介導之轉化及脂質體介導之轉化。

【0111】 在一實施例中，宿主細胞為經基因修飾之宿主細胞或微生物。

物。在此上下文中，宿主細胞或微生物可經基因修飾以增強表現其之多肽的表現及/或宿主細胞之PET酶活性。舉例而言，本文中所描述之多肽可用於補充已知具有PET酶活性之真菌或細菌之野生型菌株，以便改良及/或提高彼菌株之PET酶活性。

【0112】 本發明亦擴展至一種產生具有酯酶活性之多肽之方法，該方法包含：

- (a) 提供本文中所描述之多核苷酸；
- (b) 在宿主細胞培養物中表現多核苷酸，藉此產生多肽；及
- (c) 自宿主細胞培養物收集(b)中產生的多肽。

【0113】 本發明揭露內容亦擴展至產生本文中所描述之多肽的活體外方法，該方法包含(a)使本發明之核酸、卡匣或載體與活體外表現系統接觸；及(b)回收所產生多肽。活體外表現系統為熟習此項技術者所熟知，且為可商購的。

【0114】 適合之宿主細胞將為熟習此項技術者所熟悉的，且其說明性實例包括重組芽孢桿菌、重組大腸桿菌、重組假單胞菌、重組曲黴菌、重組木黴菌、重組鏈黴菌、重組酵母菌、重組畢赤酵母菌、重組棲熱菌或重組耶氏酵母菌。在一實施例中，宿主細胞為大腸桿菌。在另一實施例中，宿主細胞為芽孢桿菌。

【0115】 宿主細胞可使用將為熟習此項技術者已知的方法在適用於產生多肽之培養基中進行培育。適合之實例包括在適合之培養基中且在允許表現及/或分離酵素的條件下執行的搖瓶培養或在實驗室或工業發酵器中之小規模或大規模發酵(包括連續、分批、分批補料或固體狀態發酵)來培養細胞。培養將通常在來自商業供應商或根據所公開組合物製備(例

如，美國典型培養物保藏中心(American Type Culture Collection)之目錄)之適合營養培養基或適合於細胞生長之任何其他培養基中進行。

【0116】 在多肽分泌至營養物培養基中之情況下，可直接自培養物上清液回收多肽。相反地，可自細胞溶菌液或在滲透宿主細胞膜之後回收多肽。多肽可使用熟習此項技術者已知之任何適合之方法回收，該方法之說明性實例包括收集、離心、過濾、萃取、噴霧乾燥、蒸發或沉澱。視情況，多肽可藉由此項技術中已知之各種程序部分或完全純化以獲得大體上純的多肽，該等程序包括(但不限於)熱休克、層析(例如，離子交換、親和性、疏水性、層析聚焦及尺寸排阻)、電泳程序(例如，製備型等電聚焦)、差分溶解度(例如，硫酸銨沉澱)、SDS-PAGE或萃取。

【0117】 多肽可以純化形式單獨或與其他酵素(例如，PET酶或MHET酶或羧酯酶或具有PET酶活性之角質酶)組合使用以催化參與含聚酯材料，諸如含有聚酯之塑膠產品的降解及/或再循環之酵素性反應。本文中所描述之多肽可呈可溶形式或呈固相。特定言之，其可結合至細胞膜或脂質囊泡，或結合至合成支撐體，諸如例如呈珠粒、管柱、板形式及其類似形式之玻璃、塑膠、聚合物、濾膜。

【0118】 使用固定於受質上之多肽執行本發明之方法可為方便的。當以半連續或連續方式執行本發明之方法時，使用固定化多肽可為有益的。

【0119】 在一個實施例中，本文中所描述之多肽經固定於受質上。

【0120】 多肽可使用熟習此項技術者已知之技術固定於任何適合之受質上。舉例而言，多肽可藉由離子交換、吸附(例如疏水性吸附)或共價偶合而固定於支撐樹脂上。

【0121】 在一個實施例中，多肽經固定於樹脂上。在一個實施例中，多肽經固定於離子交換樹脂上。在一個實施例中，多肽經固定於樹脂上。在另一實施例中，多肽經固定於吸附樹脂上。在另一實施例中，多肽經固定於鎳親和性樹脂上。在一個實施例中，多肽經固定於共價樹脂上。在一個實施例中，多肽經固定於離子交換樹脂上。熟習此項技術者將熟悉酵素性固定技術之一般原理，且彼原理可有利地應用於根據本發明將多肽固定於基質上之上下文中。

【0122】 用於固定多肽之適合離子交換樹脂將通常包含聚合物基質或聚合物/陶瓷混合基質。此類樹脂之實例包括但不限於CM Ceramic HyperD®離子交換層析樹脂。

【0123】 在一個實施例中，離子交換樹脂為陽離子交換樹脂。對於根據本發明之方法之操作，多肽將通常固定於支撐樹脂上且負載至管柱中。

【0124】 本發明亦擴展至組合物，其包含本文中所描述之多肽、核酸或宿主細胞。

【0125】 組合物可為液體或乾燥的，例如呈散劑形式。在一些實施例中，組合物為凍乾物。舉例而言，組合物可包含多肽、核酸及/或宿主細胞及視情況存在之賦形劑及/或試劑等。適合之賦形劑可包括常用於生物化學之緩衝劑，用於調整pH之試劑，諸如苯甲酸鈉、山梨酸鈉或抗壞血酸鈉之保存劑，防腐劑，諸如澱粉、糊精、阿拉伯膠之保護劑或穩定劑，鹽，例如山梨醇、海藻糖或乳糖之糖，甘油，聚乙二醇，聚乙烯二醇，聚丙二醇，丙二醇，諸如鈣之二價離子，諸如EDTA之螯合劑，還原劑(例如β-巰基乙醇、二硫蘇糖醇、抗壞血酸、參(2-羧基乙基)磷)，胺基

酸，諸如溶劑或水溶液之載劑以及類似物。

【0126】 在一實施例中，組合物包含本文中所描述之多肽(多肽可以經分離或至少部分純化形式存在於組合物中)。在一實施例中，組合物包含按組合物之總重量計約0.1重量%至約99.9重量%，較佳地約0.1重量%至約50重量%，較佳地約0.1重量%至約30重量%，較佳地約0.1重量%至約5重量%之量的本文中所描述之多肽。在一較佳實施例中，組合物包含按組合物之總重量計約0.1至約5重量%之量的本文所描述之多肽。在另一實施例中，組合物包含按組合物之總重量計約0.1至約0.2重量%之量的本文中所描述之多肽。組合物中之多肽之量可由熟習此項技術者視例如待降解(水解)之含聚酯材料之性質及/或量及/或組合物中存在或不存在任何其他酵素/多肽而定來進行適當地調適。

【0127】 本文中所描述之組合物可進一步包含展現酵素性活性之其他多肽，不限於PET酶、酯酶、羧酯酶、MHET酶或具有混雜的PET酶/MHET酶活性之角質酶。

【0128】 在一實施例中，本文所描述之多肽與一或多種賦形劑(諸如可適當地穩定或保護多肽免於降解之賦形劑)一起溶解於含水介質中。舉例而言，本文中所描述之多肽可溶解於水中且接著與諸如甘油、山梨糖醇、糊精、澱粉、二醇(諸如丙二醇)、鹽等之賦形劑摻合。所得摻合物隨後可經乾燥以獲得散劑。用於乾燥此類混合物之方法為熟習此項技術者所熟知，且包括(但不限於)凍乾、冷凍乾燥、噴霧乾燥、超臨界乾燥、下吸式蒸發、薄層蒸發、離心蒸發、帶式乾燥、流體化床乾燥、滾筒乾燥或任何組合。

【0129】 在一實施例中，組合物包含表現本文所描述之多肽的至少

一種宿主細胞或其萃取物。「細胞萃取物」意謂藉由化學、物理及/或酵素性處理而自細胞獲得之任何部分，諸如細胞上清液、細胞碎片、細胞壁、DNA萃取物、酵素或酵素製備物或衍生自細胞之任何製備物，其基本上不含活細胞。較佳萃取物為具有酵素性活性之萃取物。組合物可包含含有本文中所描述之多肽的一或若干個宿主細胞或其萃取物，及視情況存在之一或若干個其他細胞。

【0130】 如本文中其他地方所指出，本發明人已出人意料地發現，本文中所描述之多肽(經工程化之多肽及其變體)具有更大酯酶活性，亦即當與現有PET酶及具有PET酶活性之角質酶相比時，對苯二甲酸單-酯及對苯二甲酸二-酯之水解。因此，本文中揭示一種水解對苯二甲酸單-酯及對苯二甲酸二-酯的方法，該方法包含在條件下使對苯二甲酸酯暴露於本文中所描述之多肽、組合物或宿主細胞，該等條件足以使得多肽能夠將對苯二甲酸單酯轉化成對苯二甲酸及醇；將對苯二甲酸二酯轉化成對苯二甲酸單酯及醇；或將對苯二甲酸二酯轉化成對苯二甲酸及醇。本發明亦擴展至一種降解包含聚酯之塑膠產品的方法，該方法包含使塑膠產品暴露於本文中所描述之多肽、組合物或宿主細胞。

【0131】 本發明擴展至本文中所描述之多肽、組合物或宿主細胞在以下製程中的用途：用於在好氧或厭氧條件下降解聚酯及/或使含聚酯材料作為由聚酯製成或含有聚酯之塑膠產品再循環及/或產生含有聚酯之生物可降解塑膠產品。此類方法及用途尤其適用於降解包含PET之塑膠產品。

【0132】 有利地，含聚酯材料之聚酯解聚合成單體及/或寡聚物。在一實施例中，至少一種聚酯經降解以產生可再聚合單體及/或寡聚物，其

有利地擷取或回收以用於進一步使用。

【0133】如本文中其他處所指出，塑膠產品可包含選自由以下組成之群的至少一種聚酯：聚乳酸(PLA)、聚對苯二甲酸丙二酯(PTT)、聚對苯二甲酸丁二酯(PBT)、聚異山梨醇對苯二甲酸乙二酯(PEIT)、聚對苯二甲酸乙二酯(PET)、聚羥基烷酸酯(PHA)、聚丁二酸丁二酯(PBS)、聚丁二酸己二酸丁二酯(PBSA)、聚己二酸對苯二甲酸丁二酯(PBAT)、聚呋喃酸乙二酯(PEF)、聚己內酯(PCL)、聚(己二酸乙二酯)(PEA)及前述任一者之組合。塑膠產品可包含選自由以下組成之群的至少一種聚合物：聚丙烯、聚苯乙烯、聚氯乙烯、合成橡膠、苯酚甲醛樹脂(或人造樹膠(Bakelite))、氯丁橡膠、耐綸、聚丙烯腈、PVB及聚矽氧。

【0134】降解含聚酯之材料所需的時間可視含聚酯材料自身(亦即，塑膠產品之性質及來源、其組成、形狀等)、所使用多肽之類型及量以及各種過程參數(亦即，溫度、pH、其他試劑等)而變化。熟習此項技術者可容易地使過程參數適於含聚酯材料。

【0135】有利地，降解過程係在約10℃至約80℃，較佳地約20℃至約80℃，較佳地約30℃至約80℃，較佳地約40℃至約80℃，較佳地約50℃至約80℃，甚至較佳地約60℃至約80℃，甚至更佳地約60℃至約70℃，甚至更佳地約60℃的溫度下實施。如熟習此項技術者將瞭解，溫度通常維持在活化溫度下，該活化溫度對應於多肽具有活性及/或重組微生物確實合成、產生或釋放本文所描述之多肽的溫度。在一實施例中，溫度維持在低於含聚酯材料中之聚酯的玻璃轉化溫度(T_g)。在一實施例中，降解過程或方法係在約10℃至約80℃，較佳地約20℃至約80℃，較佳地約30℃至約80℃，較佳地約40℃至約80℃，較佳地約50℃至約80℃、甚

至較佳地約60℃至約80℃、甚至更佳地約60℃至約70℃、甚至更佳地約60℃的溫度下實施。該過程或方法可適當地以連續方式、在多肽可使用若干次及/或再循環之溫度下實施。

【0136】有利地，降解過程或方法係在約5至約11的範圍內，較佳地在約6至約10的範圍內之pH下，較佳地在約6.5至約9，較佳地在約7至約9的範圍內，較佳地在約7至約8的範圍內之pH下，較佳地在約9.5至約11之pH下實施。

【0137】在一實施例中，含聚酯材料可在與多肽接觸之前經預處理以便以物理或化學方式改變其結構，以便利用一或多種酵素改良對聚酯及其中間物之存取。

【0138】由解聚合或降解過程或方法產生之單體可適當地經依序或連續地回收。視起始含聚酯材料而定，可回收單一類型之單體或若干不同類型之單體。

【0139】所回收單體可使用任何適合之純化方法進一步純化且調節成可再聚合形式。適合之純化方法之說明性實例包括組合或不組合的汽提製程、藉由水溶液分離、蒸汽選擇性冷凝、在生物製程之後對介質進行過濾及濃縮、分離、蒸餾、真空蒸發、萃取、電滲析、吸附、離子交換、沉澱、結晶、濃縮及加酸脫水及沉澱、奈米過濾、酸催化劑處理、半連續模式蒸餾或連續模式蒸餾、溶劑萃取、蒸發濃縮、蒸發結晶、液/液萃取、氫化、共沸蒸餾製程、吸附、管柱層析、簡單真空蒸餾及微過濾。

【0140】可再聚合單體可用於合成新聚酯。有利地，再聚合出具有相同性質的聚酯。然而，有可能將所回收單體與其他單體混合，例如以便合成新共聚物。替代地，所回收單體可用作化學中間物以便產生所關注的

新化合物。

【0141】 本發明亦擴展至一種包含塑膠化合物及該多肽之組合物，及/或表現該多肽之宿主細胞或其含有該多肽之萃取物。

【0142】 本發明亦擴展至包含該多肽之母料組合物、組合物及/或表現該多肽之宿主細胞或其含有該多肽之萃取物。

【0143】 有利地，本文中所描述之此類塑膠化合物或母料組合物可用於產生含聚酯材料。

【0144】 在一實施例中，所得塑膠化合物或母料組合物為順應熟習此項技術者已知之相關標準及/或標籤中之至少一者的可生物降解塑膠化合物或母料組合物，諸如標準 EN 13432、標準 ASTM D6400、OK Biodegradation Soil (Label Vincotte)、OK Biodegradation Water (Label Vincotte)、OK Compost (Label Vincotte)、OK Home Compost (Label Vincotte)。

【0145】 有利地，含聚酯材料(亦即，塑膠化合物或母料組合物或塑膠產品)之降解過程係在約10°C至約80°C，較佳地約20°C至約80°C，較佳地約30°C至約80°C，較佳地約40°C至約80°C，較佳地約50°C至約80°C，甚至較佳地約60°C至約80°C，甚至更佳地約60°C至約70°C，甚至更佳地約60°C +/-5°C的溫度下實施。

【0146】 替代地，含聚酯材料(亦即塑膠化合物、母料組合物或塑膠產品)之降解過程係在約50°C至約70°C，更佳地60°C +/-5°C之溫度下實施。

【0147】 本文所揭示之具有酯酶活性之工程化多肽適用於一系列應用，包括工業應用，其說明性實例包括作為添加劑用於清潔劑、飼料組合

物(包括動物飼料)、紡織物產生、電子裝置及生物醫學應用。舉例而言，本文中所揭示之具有酯酶活性之工程化多肽可用於紡織品加工或紡織品產生，其中其可用作外酯酶以適當地修飾紡織品纖維之特性。

【0148】 現將參考以下實例來描述本發明，該等實例說明本發明之一些較佳態樣。然而，應理解，本發明之以下描述之特殊性並不取代本發明之先前描述之一般性。

實例

實例1：祖先序列重構

【0149】 自 pfam 擷取屬於蛋白質家族 PF12695、PF01738 及 PF12740的所有胺基酸序列。使用CD-HIT移除冗餘以達到100%序列一致性(Fu等人，2012)且執行一對一(all-vs-all) pBLAST。所得表在cytoscape中視覺化為序列類似性網路(Shannon等人，2003)且在該網路經視覺化為prefuse-force導引之網路圖形之前刪除具有小於40%序列一致性之邊緣連接序列。自uniprot擷取屬於包括LCC、TfCut2及IsPET酶之序列集群之全部序列且使用CD-HIT移除冗余以達至95%序列一致性。利用SignalP5.0 (Almagro Armenteros等人，2019)識別任何革蘭氏陽性或革蘭氏陰性之信號肽且手動地移除。使用MAFFT中之GINSI協定(Katoh及Standley 2013)產生此資料集之多序列比對，根據所解析晶體結構手動地編輯該多序列比對。最終比對具有397個比對序列。

【0150】 使用預設樹搜尋參數在IQ-TREE (Minh等人2020)中執行最大概似(ML)樹搜尋及親緣關係(phylogenetic)重構的100次獨立重複。在ModelFinder (Kalyaanamoorthy等人2017)中藉由艾凱克及貝氏(Akaike and Bayesian)資訊準則識別最佳擬合(WAG+F+R8) (Whelan及Goldman

2001)之序列演化模型。對各推論之分支支持係以1000次重複之替代概似比檢定統計數據(Anisimova及Gascuel 2006)及超快自舉近似(ultrafast bootstrap approximation) (ufboot) (Hoang等人2018)計算。因為10000次重複進行之近似無偏(AU) (Shimodaira 2002)測試在統計學上可能低於任何其他者而不能拒絕任何單一拓樸，因此取樣代表完整資料集內大部分拓樸多樣性之20種收斂拓樸用於ASR。使用序列演化模型WAG+G4在來自PAML (Yang 2007)套件之CodeML中藉由ML在20種拓樸內重構祖先序列。已在現存序列中識別之18種保守性插入以離散二項式特質處理(1為插入存在，0為插入不存在)，且使用二元類耶克斯-康托(Jukes-Cantor-like)等比率模型，在R 套件(package) Ape (Paradis，Claude及Strimmer 2004)中藉由ML重構。在具有最小ufboot支持0.9及最小平均後驗機率0.8的20種拓樸內自LCC與TfCut2之間共享的共用節點取樣48種重構祖先序列。

實例2：蛋白質表現及純化

【0151】 質體藉由熱休克轉化成化學勝任E. cloni®細胞(Lucigen)且接種至補充有100 µg/L康黴素之潛溶型培養液(LB)瓊脂上且在37°C 培育整夜。使用單菌落以在2.2 mL 96孔深孔塊中接種補充有100 µg/mL康黴素之1.5 mL自誘導培養基且以1050 rpm在37°C 生長5小時，然後在室溫(RT；25°C)生長16小時。

【0152】 藉由在RT以2000 × g離心15分鐘來採集細胞且再懸浮於溶解緩衝液(1X BugBuster®蛋白質萃取試劑(Merck-Millipore)、20 mM Tris、300 mM NaCl、1 U/ml Turbonuclease (Sigma) pH 8)中。將細胞懸浮液在RT輕輕搖動培育20分鐘。藉由在RT以2250 × g離心1小時以將溶解物與不可溶細胞碎片分離。經澄清溶解物接著用100 µl平衡緩衝液(20 mM

Tris , 300 mM NaCl pH 8)稀釋且使用在平衡緩衝液中平衡之96孔 HisPur™ Ni-NTA旋轉板(ThermoFisher Scientific)藉由帶鎳之IMAC純化，將樣本用250 µl洗滌緩衝液(20 mM Tris , 300 mM NaCl , 10 mM咪唑pH 8)洗三次且用250 µl溶離緩衝液(20 mM Tris , 300 mM NaCl , 150 mM咪唑pH 8)溶離。在添加洗滌或溶離緩衝液之後，全部離心步驟在RT以1000 × g進行1分鐘。將溶離液(eluate)儲存於4℃。

實例3：偵測MOCT及DOCT之水解的HPLC活性分析

【0153】 用1.5 mM受質、5% DMSO及來自Ni-NTA純化之溶離液的1:10稀釋液在反應緩衝液(45 mM NaH₂PO₄ , 90 mM NaCl , pH 7.5)中進行針對對苯二甲酸單辛酯(MOCT)或對苯二甲酸二辛酯(DOCT)之酵素性活性分析。

【0154】 將反應物在50℃培育64分鐘，接著藉由在95℃下加熱至少10分鐘進行淬滅。使用高效液相層析(HPLC)分析反應物且與不含酵素之對照反應物進行比較。

【0155】 藉由與使用合成或商用標準品產生之校準曲線進行比較來測定對苯二甲酸及對苯二甲酸單辛酯之濃度。

【0156】 為比較所有變體之活性，使用在254 nm下之吸光度。使用TPA標準品(Sigma Aldrich)獲得HPLC方法之TPA的滯留時間及對應校準曲線。為表徵選定變體之MOCT及DOCT水解活性，對基於與標準樣本之比較的對應於TPA之峰進行積分以獲得峰面積。展示MOCT及DOCT水解活性之選定變體展示於表1中。

【0157】 具有SEQ ID NO: 32及34之多肽似乎具有針對DOCT比MOCT更高之活性，而SEQ ID NO: 44-54似乎具有針對MOCT比DOCT顯

著更高之活性。SEQ ID NO: 6-8顯示高效DOCT水解，而SEQ ID NO: 9-11顯示高效DOCT水解(參看表2至3)。

【0158】 顯示水解MOCT及DOCT之活性的SEQ ID NO: 1-118的分析指示該等序列各自屬於共同序列-SEQ ID NO: 119。

表1：例示性MOCT酶及DOCT酶酵素之胺基酸序列

SEQ ID NO	序列
1	MAANPYERGPDPTEASLEASSGPFSVSETSVSRLSASGFGGGTIYYPTTTSSGT YGAVAIISPGYTATQSSIAWLGPRLASHGFVVITIDTNTTFDQPDSRARQLMAA LNYLVNRSSVRSRIDSSRLAVMGHSMGGGGTLRAAEDNPSLKAAIPLTPWHT NKNWSSVRVPTLIIGAENDTIAPVSSHAKPFYNSLPSSTPKAYLELNGASHFAP NSSNTTIGKYSVSWLKRFVDNDTRYSQFLCPAPHDDSAISEYRSTCPY
2	MAENPYERGPDPTESSIEALRGPFVAVSEESVSRLSVSGFGGGTIYYPTDTSEGT FGAVAIISPGYTGTQSSMAWLGPRIASQGFVVFTIDTNTTYDQPDSRARQLQA ALDYLVEDSSVRDRIDPNRLGVMGHSMGGGGTLRAAEDRPDLKAAIPLTPW HLNKNWSSVRVPTLIIGAENDTIAPVRTHAEPFYESLPSSLDKAYLELDGASHF APNISNTTIAKYSISWLKRFVDDDTRYEQFLCPPPRTGSEISEYRSTCPF
3	MAENPYERGPDPTEASIEAERGPFAIAQVSVPAAGSGSGFGGGTIYYPTDTSQG TFGAVAIISPGFTATEASIAWLGPRLASQGFVVITIDTNSRYDQPSARADQLLA ALDYLTQSSSVRSRIDPNRLAVMGHSMGGGGTLEAAENRPSLKAAIPLAPWN TNKNWSSVRVPTMIIGAQNNTIAPVGSHAEPFYNSLPASPEKAYLELNGADHF APTSSNTTIAKYSISWLKRFVDDDTRYDQFLCPAPSPDSAISEYRSTCPH
4	MQANPYQRGPDPPTSASLEASSGPFSVSTTSVSRLSASGFGGGTIYYPTTTSSGK YGAVAIISPGYTATQSSIAWLGRRLASHGFVVITIDTNTTLDQPDSRATQLMAA LNYVVNSSTVRSRVDASRLAVMGHSMGGGGTLIAAENNPSTLKAAIPLTPWH TSKNFSSVRVPTLIIGAENDTIAPVSSHAKPFYNSLPSTTSKAYLELNGASHFAP NSSNTPIGKYSISWMKRFVDNDTRYSPFLCGAPHQGA VISEYRDNCPY
5	MAANPYERGPNPTEALLEARSGPFSVSEERASRLGADGFGGGTIYYPRENSD NTYGAVAIISPGYTGTQASVAWLGERIASHGFFVITIDTNTTLDQPDSRARQLN AALDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPW HLNKNWSSVRVPTLIIGADLDTIAPVLTHAEPFYNSLPSSISKAYLELDGATHF APNITNKTIGKYSVAWLKRFVDEDTRYTQFLCPGPRTGSDVEEYRSTCPF
6	MAANPYERGPNPTDALLEARSGPFSVSEENVSRLSASGFGGGTIYYPRENSEN TYGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNA ALDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELDGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
7	MAANPYERGPNPTDALLEARSGPFSVSEENVSRLSASGFGGGTIYYPRENSEN TYGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNA ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVTVPPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELDGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
8	MAANPYERGPNPTDALLEARSGPFSVSEENVSRLSASGFGGGTIYYPRENSEN TYGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNA

	ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELDGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
9	MAANPYERGPNPSTDALLEARSGPFSVSEENVSRLSASGFGGGTIYYPRENSEN TYGAVAIISPGYTGTAASIAWLGERIASHGFFVITIDTNTTLDQPDSSRAEQLNA ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELDGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
10	MAANPYERGPNPSTDALLEARSGPFSVSEENVSRLSASGFGGGTIYYPRENSSN TYGAVAIISPGYTGTEASIAWLGERIASHGFFVITIDTITTLTLDQPDSSRAEQLNAA LNHMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVTVPPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELDGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
11	MAANPYERGPNPSTDALLEARSGPFSVSEENVSRLSASGFGGGTIYYPRENSEN TYGAVAIISPGYTGTEASIAWLGERIASHGFFVITIDTITTLTLDQPDSSRAEQLNAA LNHMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVTVPPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELDGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
12	MAANPYERGPDPTESSLEASSGPFSVSETSVSRLSASGFGGGTIYYPTTTSEGT YGAVAIISPGYTATQSSIAWLGPRLASHGFFVITIDTNTTFDQPDSSRARQLMAA LNYLVNRSSVRSRIDSSRLAVMGHSMGGGGTLRAAEDNPSLKA IPLTPWHT NKNWSSVRVPTLIIGAENDTIAPVSSHAKPFYNSLPSSSTPKAYLELNGASHFAP NSSNTTIGKYSIAWLKRFVDNDTRYSQFLCPAPHDDSAISEYRSTCPY
13	MAANPYERGPDPTESSLEASSGPFSVSQTSVSRLSVSGFGGGTIYYPTDTSSGT YGAVAIISPGYTATQSSIAWLGPRLASHGFFVITIDTNSTFDQPDSSRARQLMAA LDYLVNRSPVRSRIDSSRLAVMGHSMGGGGTLRAAEDNPSLKA IPLTPWHT DKNWSSVRVPTLIIGAENDTIAPVSSHAKPFYNSLPSSSTSKAYLELNGASHFAP NSSNTTIGKYSVSWLKRFDNDTRYSQFLCPAPHDDSAISEYRSTCPY
14	MADNPYERGPAPTESSIEASRGPFVVSQTSVSSLSVSGFGGGTIYYPTSTSEGT FGAVAIISPGYTATQSSIAWLGPRLASQGFVVFTIDTNTRYDQPDSSRGRQLLAA LDYLTQRSSVRSRIDASRLGVMGHSMGGGGTLEAAEDRPSLQA IPLTPWNL DKNWSSVRVPTMIIGAENDTIAPVSSHSEPFYTSPLSSLDKAYLELNGASHFAP NTSNTTIKYSISWLRFDNDTRYEQFLCPAPSRDITISEYRDTCPH
15	MADNPYERGPDPTESSIEASRGPFVVSQTSVSRLSVSGFGGGTIYYPTSTSEGT FGAVAIISPGYTATQSSIAWLGPRLASQGFVVFTIDTNTRYDQPDSSRGDQLLAA LDYLTQRSSVRSRIDPSRLAVMGHSMGGGGTLEAAKDRPSLKA IPLTPWNT DKNWSSVTVPPTLIIGAENDTIAPVSSHSPKFYNSLPSSPEKAYLELNGASHFAP NSSNTTIKYSISIAWLKRFVDNDTRYSQFLCPAPSADSAISEYRDTCPY
16	MQANPYQRGPDPTSSSLEASSGPFSVSTTSVSRLSVSGFGGGTIYYPTNTSSGT YGAVAIISPGYTATQSSIAWLGPRLASHGFFVITIDTNSTFDQPDSSRATQLMAA LDYVVNSSPVRSRVDSSRLAVMGHSMGGGGTLRAAEDNPSLKA IPLTPWH TDKNFSSVRVPTLIIGAENDTVAPVSSHAKPFYNSLPSSSTPKAYLELNGASHFA PNSSNTTIGKYSVSWMKRFVDNDTRYSQFLCPAPHDDSAISEYRSTCPY
17	MQANPYQRGPDPTESSEASSGPFSVSTTSVSRLSVSGFGGGTIYYPTSTSSGT YGAVAIISPGYTATQSSIAWLGPRLASHGFFVITIDTNSTFDQPDSSRATQLMAA LNYLVNSSVRSRIDSSRLAVMGHSMGGGGTLRAAEDNPSLKA IPLTPWHT DKNFSSVTVPPTLIIGAENDTIAPVSSHAKPFYNSLPSSSTSKAYVELNGASHFAP NSSNTTIGKYSVSWMKRFVDNDTRYSQFLCGAPHDDSAISEYRSNCPY
18	MQANPYQRGPDPTSASLEASSGPFSVSTTSVSRLSASGFGGGTIYYPTTTSSGT YGAVAIISPGYTATQSSIAWLGPRLASHGFFVITIDTNSTFDQPDSSRATQLMAA

	LNYVVNSSTVRSRVDSSRLAVMGHSMGGGGTLQAAEDNPSLKA A I P L T P W H T N K N F S S V R V P T L I I G A E N D T I A P V S S H A K P F Y N S L P S S T S K A Y V E L N G A S H F A P N S S N T T I G K Y S V S W M K R F V D N D T R Y S Q F L C G A P H D D S A I S E Y R S T C P Y
19	MAANPYERGPDPPTNASLEASSGPFSVSETSVSRLSASGFGGGGTIYYPTSTSEGT YGAVAIISPGYTGTQSSIAWLGPRLASHGFVVITIDTNTTLDQPDSRASQLMAA LNYLVNRSTVRSRIDASRLAVMGHSMGGGGTLRAAEQRPSLKA A I P L T P W H T DKNWSSVRVPTLIIGAENDTIAPVSSHAKPFYNSLPSTISKAYLELNGASHFAP N S S N T T I G K Y S I S W L K R F V D N D T R Y S Q F L C P A P R Q G A L I E E Y R D T C P Y
20	MQANPYQRGPDPPTSSSLEASSGPFSVSTTSVSSLSVSGFGGGGTIYYPTNTSSGT YGAVAIISPGYTATQSSIAWLGPRLASHGFVVITIDTNSTFDQPDSRARQLMAA LNYLVNRSPVRSRVDSSRLAVMGHSMGGGGTLRAAEDNPSLKA A I P L T P W H T D K N F S S V R V P T L I I G A E N D T I A P V S S H A K P F Y N S L P S S T S K A Y L E L N G A S H F A P N S S N T T I G K Y S V S W M K R F V D N D T R Y S Q F L C P A P H D D S A I S E Y R D T C P Y
21	MAANPYERGPDPTESSLEASSGPFSVSTTSVSRLSVSGFGGGGTIYYPTSTSEGT YGAVAIISPGYTATQSSIAWLGRRLASHGFVVITIDTNTTFDQPDSRADQLMAA LNYLVNRSSVRSRIDSSRLAVMGHSMGGGGTLRAAKDRPSLKA A I P L T P W H T DKNWSSVTVP T L I I G A E N D T I A P V S S H A K P F Y N S L P S S T S K A Y L E L N G A S H F A P N S S N T T I G K Y S V A W L K R F V D N D T R Y S Q F L C P A P H D G S A I S E Y R D T C P Y
22	MQANPYQRGPDPPTSASLEASSGPFSVSTTSVSRLSASGFGGGGTIYYPTSTSSGT YGAVAIISPGYTATQSSIAWLGRRLASHGFVVITIDTNTTLDQPDSRATQLMAA LNYLVNRSTVRSRIDASRLAVMGHSMGGGGTLRAAEQNPSLKA A I P L T P W H T DKNFSSVRVPTLIIGAENDTIAPVSSHAKPFYNSLPSTTSKAYLELNGASHFAP N S S N T P I G K Y S I S W M K R F V D N D T R Y S Q F L C G A P H Q G A V I S E Y R D N C P Y
23	MAENPYERGPDPTESSIEATRGPFAVSQTSVSRLSVSGFGGGGTIYYPTSTSEGT FGAVAIISPGYTATQSSIAWLGPRLASQGFVVITIDTNSRYDQPASRGDQLLAA LDYLTQSSSVRSRIDPSRLAVMGHSMGGGGTLEAAKDRPSLKA A I P L T P W N T DKNWSEVRVPTLIIGAENDTVAPVSSHAEPFYNSLPSSPEKAYLELNGASHFA P N S S N T T I A K Y S I S W L K R F V D D D T R Y D Q F L C P A P S P D S A I S E Y R S T C P H
24	MAANPYERGPDPTEASLEASSGPFSVSQTSVSSLSVSGFGGGGTIYYPTSTSSGT YGAVAIISPGYTATQSSIAWLGPRLASHGFVVITIDTNSTFDQPDSRARQLMAA LDYLVNRSSVRSRIDSSRLAVMGHSMGGGGTLRAAEDRPSLKA A I P L T P W H T NKNWSSVRVPTLIIGAENDTIAPVSSHAKPFYNSLPSSTSKAYLELNGASHFAP N S S N T T I G K Y S V S W L K R F V D N D T R Y S Q F L C P A P H D D S A I S E Y R D T C P Y
25	MAANPYERGPDPTEASLEASSGPFSVSETSVSRLSASGFGGGGTIYYPTTTSEGT YGAVAIISPGYTATQSSIAWLGPRLASHGFVVITIDTNSTFDQPDSRADQLMAA LNYLVNRSSVRSRIDSSRLAVMGHSMGGGGTLQAAEDRPSLKA A I P L T P W H T NKNWSSVRVPTLIIGAENDTIAPVSSHAKPFYNSLPSSTSKAYLELNGASHFAP N S S N T T I G K Y S V S W L K R F V D N D T R Y S Q F L C P A P H D D S A I S E Y R S T C P Y
26	MQANPYQRGPDPPTSSSLEASSGPFSVSTTSVSRLSASGFGGGGTIYYPTTTSSGT YGAVAIISPGYTATQSSIAWLGRRLASHGFVVITIDTNSTFDQPDSRATQLMAA LNYLVNSSTVRSRIDASRLAVMGHSMGGGGTLRAAEDNPSLKA A I P L T P W H T DKNFSSVRVPTLIIGAENDTIAPVSTHAKPFYNSLPSSTSKAYVELNGASHFAP N S S N T P I G K Y S I S W M K R F V D N D T R Y S Q F L C G A P H D G S A I S E Y R D N C P Y
27	MADNPYERGPAPTEASIEASRGPF AISQVSVPASGSGFGGGGTIYYPTDTSQGT FGAVAIISPGFTATEASIAWLGPRLASQGFVVITIDTNSRYDQPDSRGKQLLAA LDYLTQKSSVRSRIDPNRLAVMGHSMGGGGTLEAAENRPSLKA A I P L T P W H T DKNWSSVRVPTMIIGAENDTIAPVGSHAEPFYNSLPSSPEKAYLELKGADHFA P T S S N T T I A K Y S I S W L K R F V D D D T R Y D Q F L C P A P S S D S A I S E Y R D T C P H
28	MAENPYERGPAPTESSIEATRGPFAVSQTSVSSLSVSGFGGGGTIYYPTSTSEGT FGAVAIISPGYTASQSSIAWLGPRLASQGFVVFTIDTNTRYDQPASRGDQLLAA

	LDYLTQRSSVRSRIDASRLGVMGHSMGGGGTLEAAKDRPSLQAAIPLTGWNL DKNWSEVRVPTLVIGAENDTIAPVSSHSEPFYNLSLPSSLDKAYLELNGASHFA PNSSNTTIAKYSISWLKRFDNDTRYEQFLCPAPRPGSTIEEYRDTCPH
29	MAANPYERGPDPTESSLEASSGPFSVSQTSVSRLSVSGFGGGGTIYYPTSTSSGT YGAVAIISPGYTATQSSIAWLGPRLASHGFVVITIDTNSTFDQPDSRADQLMAA LNYLVNRSSVRSRIDSSRLAVMGHSMGGGGTLRAAEDRPSLKAACIPLTPWHT DKNWSSVTVP TLIIIGAENDTIAPVSSHAKPFYNLSLPSSSTKAYLELNGASHFAP NSSNTTIGKYSISWLKRFDNDTRYEQFLCPAPHDDSAISEYRSTCPY
30	MAANPYERGPDPTESSLEASSGPFSVSETSVSRLSVSGFGGGGTIYYPTSTSSGT YGAVAIISPGYTATQSSIAWLGPRLASHGFVVITIDTNSTFDQPDSRARQLMAA LDYLVNSSSVRSRIDSSRLAVMGHSMGGGGTLRAAEDRPSLKAACIPLTPWHT NKNWSSVRVPTLIIIGAENDTVAPVSSHAKPFYNLSLPSSSTPKAYLELNGASHFA PNSSNTTIGKYSVAWLKRFDNDTRYEQFLCPAPHDDSAISEYRSTCPY
31	MAANPYERGPDPTESSLEASSGPFSVSETSVSRLSVSGFGGGGTIYYPTDTSEGT YGAVAIISPGYTATQSSIAWLGPRLASHGFVVITIDTNSTFDQPDSRARQLQAA LNYLVNSSSVRSRIDSNRLAVMGHSMGGGGTLRAAEDNPSLKAACIPLTPWHT NKNWSSVTVP TLIIIGAENDTIAPVSSHAKPFYNLSLPSSSTKAYLELNGASHFAP NSSNTTIGKYSISWLKRFDNDTRYEQFLCPAPHDDSAISEYRSTCPY
32	MQANPYQRGPDPTESSLEASSGPFSVSTTSVSRLSVSGFGGGGTIYYPTTTSSGT YGAVAIISPGYTATQSSIAWLGPRLASHGFVVITIDTNSTFDQPDSRATQLMAA LNYVVNSSPVRSRVDDSSRLAVMGHSMGGGGTLRAAEDNPSLKAACIPLTPWH TNKNFSSVRVPTLIIIGAENDTVAPVSSHAKPFYNLSLPSSSTPKAYLELNGASHFA PNSSNTTIGKYSVAWMKRFDNDTRYSPFLCGAPHDDSAISEYRSTCPY
33	MAANPYERGPDPTEASLEASSGPFSVSTTSVSRLSVSGFGGGGTIYYPTSTSEGT YGAVAIISPGYTATQSSIAWLGRRLASHGFVVITIDTNSTFDQPDSRADQLMAA LNYLVNRSSVRSRIDSSRLAVMGHSMGGGGTLRAAKDRPSLKAACIPLTPWHT DKNWSSVRVPTLIIIGAENDTIAPVSSHAKPFYNLSLPSSSTKAYLELNGASHFAP NSSNTTIGKYSVSWLKRFDNDTRYEQFLCPAPHDDSAISEYRDTCPY
34	MAENPYERGPAPTESSIEASRGPFVVSQTSVSRLSVSGFGGGGTIYYPTSTSEGT GAVAIISPGYTATQSSIAWLGPRLASQGFVVFTIDTNTRYDQPASRGDQLLAAL DYLTQRSSVRSRIDASRLGVMGHSMGGGGTLEAAKDRPSLQAAIPLTPWNLD KNWSEVRVPTLIIIGAENDTIAPVSSHSEPFYNLSLPSSLDKAYLELNGASHFAPN SSNTTIAKYSISWLKRFDNDTRYEQFLCPAPSPDSTISEYRDTCPH
35	MAANPYERGPDPTESSLEASSGPFSVSEENVSRSLASGFGGGGTIYYPRENSEN TYGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNNTLDQPDSRAEQLNA ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAACIPLTPWH LNKNWSSVTVP TLIIIGADLDTIAPVATHAKPFYNLSLPSSISKAYLELDGATHFA PNIPNKIIGKYSVAWLKRFDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
36	MAANPYERGPDPTESSLEASSGPFSVSETSVSRLSVSGFGGGGTIYYPTSTSSGT YGAVAIISPGYTATQSSIAWLGPRLASHGFVVITIDTNSTFDQPDSRARQLMAA LNYLVNSSSVRSRIDSSRLAVMGHSMGGGGTLRAAEDNPSLKAACIPLTPWHT DKNWSSVRVPTLIIIGAENDTIAPVSSHAKPFYNLSLPSSSTPKAYLELNGASHFAP NSSNTTIGKYSISWLKRFDNDTRYEQFLCPAPHDDSAISEYRSTCPY
37	MAANPYERGPDPTEASLEATSGPFVSSETSVSRLSVSGFGGGGTIYYPTDTSSGT YGAVAIISPGYTATQSSIAWLGPRLASHGFVVITIDTNSTFDQPDSRARQLMAA LNYLVNSSPVRSRIDSSRLAVMGHSMGGGGTLRAAEDNPSLKAACIPLTPWHT NKNWSSVRVPTLIIIGAENDTIAPVSSHAKPFYNLSLPSSSTKAYLELNGASHFAP NSSNTTIGKYSISWLKRFDNDTRYEQFLCPAPHDGSASEYRSTCPY
38	MAANPYERGPDPTESSLEASSGPFSVSEENVSRSLASGFGGGGTIYYPRENSDN TYGAVAIISPGYTGTASIAWLGERIASHGFFVITIDTITLDQPDSRAEQLNAA

	LNHMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVTVP TLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELDGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
39	MAANPYERGP NPTDALLEARSGPFSVSEERASRLGADGFGGGTIYYPRENSD NTYGAV AISP GYTGTQASVAWLGERIASHG FVVITIDTNTTLDQPDSRARQLN AALDY MINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPW HLNKNWSSVRVP TLIIGADLDTIAPVLTHAKPFYNSLPSSISKAYLELDGATHF APNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
40	MAANPYERGP NPTDALLEARSGPFSVSEENVSRLSASGFGGGTIYYPRENSSN TYGAV AISP GYTGTQASIAWLGERIASHG FVVITIDTNTTLDQPDSRAEQLNA ALDYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVTVP TLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELDGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
41	MAANPYERGP NPTDALLEARSGPFSVSEENVSRLSASGFGGGTIYYPRENSSN TYGAV AISP GYTGTQASIAWLGERIASHG FVVITIDTNTTLDQPDSRARQLNA ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVRVP TLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELDGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
42	MAANPYERGP DPTESSLEASSGPFSVSETSVSRLSASGFGGGTIYYPTTTSEGT YGAV AISP GYTGTQSSIAWLG PRLASHG FVVITIDTNTTFDQPDSRARQLMAA LNYLVNRSSVRSRIDASRLAVMGHSMGGGGTLRAAEQRPSLKAAIPLTPWHT DKNWSSVRVP TLIIGAENDTIAPVSTHAKPFYNSLPSSISKAYLELNGASHFAP NTSNTTIGKYSISWLKRFVDNDTRYTQFLCPAPRDGSAIEEYRDTCPY
43	MAANPYERGP NPTDALLEARSGPFSVSEENVSRLSASGFGGGTIYYPRENSDN TYGAV AISP GYTGTQASIAWLGERIASHG FVVITIDTNTTLDQPDSRARQLNA ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVRVP TLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELDGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
44	MAENPYERGP APTESSIEASSGPFSVSTTSVSSLVSGFGGGTIYYPTSTSEGT GAV AISP GYTATQSSIAWLG RRLASHG FVVITIDTNSTYDQPDSRGDQLLAAL DYLTQRSSVRSRIDPSRLAVMGHSMGGGGTLEAAKDRPSLKAAIPLTPWHTD KNWSSVRVP TLIIGAENDTIAPVSSHAIPFYNSLPSSTEKAYLELNGASHFAPN SSNTTIGKYSVSWLKRFVDNDTRYSQFLCPAPSPDSAISEYRDTCPH
45	MADNPYERGP DPTESSIEASRGPF AVSETSVSRLSVSGFGGGTIYYPTTTSEGT FGAV AISP GYTGTQSSIAWLG PRLASQG FVVITIDTNTTYDQPDSRARQLLAA LDYLTQRSSVRSRIDASRLAVMGHSMGGGGTLRAAEDRPSLKAAIPLTPWHT DKNWSSVRVP TLIIGAENDTIAPVSSHAEPFYNSLPSSLPKAYLELNGASHFAP NTSNTTI AKYSISWLKRFVDNDTRYTQFLCPAPRPGSAIEEYRDTCPY
46	MADNPYERGP APTESSIEASRGPF AVSQTSVSSLVSGFGGGTIYYPTSTSEGT FGAV AISP GYTATQSSIAWLG PRLASQG FVVFTIDTNTRYDQPDSRGRQLLAA LDYLTQRSSVRSRIDPSRLGVMGHSMGGGGTLEAAKDRPSLQAAIPLTPWNL DKNWSSVRVP TLIIGAENDTIAPVASHSEPFYNSLPSSLDKAYLELNGASHFAP NSSNTTI AKYSISWLKRFIDNDTRYEQFLCPAPSRDSTISEYRDTCPH
47	MQANPYQRGPDPTSSSLEASSGPFSVSTTSVSRLSVSGFGGGTIYYPTNTSSGT YGAV AISP GYTATQSSIAWLG PRLASHG FVVITIDTNSTFDQPDSRATQLMAA LNYVVNSSPVRSRVDSNRLAVMGHSMGGGGTLRAAEDNPSLKAAIPLTPWH TNKNFSSVRVP TLIIGAENDTIAPVSSHAKPFYNSLPSSSTSKAYLELNGASHFA PNSSNTTIGKYSVSWMKRFVDNDTRYSQFLCPAPHDDSAISEYRSTCPY
48	MAENPYERGP DPTESSIEASRGPF AVSQTSVSSLVSGFGGGTIYYPTSTSEGT GAV AISP GYTATQSSIAWLG PRLASQG FVVFTIDTNTRYDQPASRGRQLLAAL

	DYLTQRSSVRSRIDPSRLAVAGHSMGGGGTLEAAEDRPSLQAAIPLAPWNLD KNWSSVRVPTLIIGGESDTVAPVSSHSEPFYNLSLSPSPDKAYLELNNASHFFPN TSNTTMAKYMISWLKRFDNDTRYEQFLCPAPSRDSTISEYRDTCPH
49	MAENPYERGPDPTEASIEASRGPFASQVSVPSGSGSGFGGGGTIIYYPTDTSQGT FGAVAIISPGFTATEASIAWLGPRLASQGFVVITIDTNSRYDQPDARADQLLAA LDYLTQKSSVRSRIDPNRLAVMGHSMGGGGTLEAAENRPSLKA AIPLAPWNT NKNWSSVRVPTMIIGAQN DTIAPVGS SHAEPFYNLSLPASPEKAYLELKGADHF APTSPNTTIAKYSISWLKRFDVDDTRYDQFLCPAPSSDSAISEYRDTCPH
50	MAENPYERGPAPTESSIEASRGPFASQTSVSSLSVSGFGGGGTIIYYPTSTSEGTF GAVAIISPGYTATQSSIAWLGPRLASQGFVVITIDTNSRYDQPASRGDQLLAA DYLTQRSSVRSRIDPSRLAVMGHSMGGGGTLEAAKDRPSLKA AIPLTPWNTD KNWSSVRVPTLIIGAENDTIAPVSSHAEPFYNLSLPSSPEKAYLELNGASHFAPN SSNTTIAKYSISWLKRFDNDTRYEQFLCPAPSPDSTISEYRDTCPH
51	MAENPYERGPDPTESSIEATRGPFAVSQTSVSSLSVSGFGGGGTIIYYPTSTSEGT FGAVAIISPGYTATQSSIAWLGPRLASQGFVVFTIDTNTRYDQPASRGDQLLAA LDYLTQRSSVRSRIDASRLAVMGHSMGGGGTLEAAKDRPSLQAAIPLTPWNL DKNWSEVRVPTLIIGAENDTVAPVSSHSEPFYNLSLSPSPDKAYLELNGASHFA PNSSNTTIAKYSISWLKRFDVDDTRYEQFLCPAPSPDSAISEYRDTCPH
52	MQSNPYQRGPDPTRASLEASDGPFVSTTSVSSLSVSGFGGGGTIIYYPTSTSSGT FGAVAIISPGYTATQSSIAWLGRR LASHGFVVITIDTNSRFDQPASRASQLLAA NYLTNSSSVRSRVDASRLAVAGHSMGGGGTLEAAEDNPSLKA AVPLTPWNT DKNWSEVRVPTLIIGAENDTVAPVSSHAIPFYNLSLPSTEKAYVELNGASHFA PNSSNTTISKYSISWMKRFDNDTRYSQFLCGAPNQDSAISDYRTNCPH
53	MQANPYQRGPDPTSASLEASSGPFVSTSSVSSLSASGFGGGGTIIYYPTTTSSGT YGAVAIISPGYTATQSSIAWLGRR LASHGFVVITIDTNSTFDQPDSRATQLMAA LNYVVNSSTVRSRVDASRLAVMGHSMGGGGTLIAEDNPSLKA AIPLTPWH TSKNFSSVRVPTLIIGAENDTIAPVSSHAKPFYNLSLPSTSKAYVELNGASHFAP NSSNTPIGKYSISWMKRFDNDTRYSPFLCGAPHQGA AISEYRDNC PY
54	MADNPYERGPDPTESSIEASRGPFASQTSVSSLSVSGFGGGGTIIYYPTDTSEGT FGAVAIISPGYTASQSSIAWLGPRLASQGFVVITIDTNSRYDQPDSRGRQLLAA LDYLTQRSSVRSRIDPNRLAVMGHSMGGGGTLEAAEDRPSLKA AIPLTPWHT DKNWSEVRVPTMIIGAENDTIAPVSSHAEPFYNLSLPSSPEKAYLELNGASHFA PNTSNTTIAKYSISWLKRFDVDDTRYDQFLCPAPSPDSAISEYRDTCPH
55	MAANPYERGPNP TDALLEARS GPFSVSEENVSRLSASGFGGGGTIIYYPRENSSN TYGAVAIISPGYTGT EASIAWLG ER IASHGFVVITIDTIT TLDQPDSRAEQLNAA LNHMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKA AIPLTPWHL NKNWSSVT VPTLIIGADLD TIAPVATHAKPFYNLSLPSSISKAYLELDGATHVAP NIPNKIIGKYSVAWLKRFDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
56	MAANPYERGPNP TDALLEARS GPFSVSEENVSRLSASGFGGGGTIIYYPRENSEN TYGAVAIISPGYTGTQASIAWLG ER IASHGFVVITIDTNT TLDQPDSRARQLNA ALDYMINDSA VRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKA AIPLTPWH LNKNWSSVRVPTLIIGADLD TIAPVATHAKPFYNLSLPSSISKAYLELDGATHV APNIPNKIIGKYSVAWLKRFDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
57	MAANPYERGPNP TDALLEARS GPFSVSEENVSRLSASGFGGGGTIIYYPRENSEN TYGAVAIISPGYTGTQASIAWLG ER IASHGFVVITIDTNT TLDQPDSRARQLNA ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKA AIPLTPWH LNKNWSSVT VPTLIIGADLD TIAPVATHAKPFYNLSLPSSISKAYLELDGATHVA PNIPNKIIGKYSVAWLKRFDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
58	MAANPYERGPNP TDALLEARS GPFSVSEENVSRLSASGFGGGGTIIYYPRENSEN TYGAVAIISPGYTGTQASIAWLG ER IASHGFVVITIDTNT TLDQPDSRARQLNA

	ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVATHAKPFYN SLPSSISKAYLELDGATHV APNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRD GGEVEEYRSTCPF
59	MAANPYERGNPTDALLEARSGPFSVSEENVSR L S A S G F G G G T I Y Y P R E N S S N TYGAV AISPGYTGT EASIAWLGERIASHGFV VITIDTIT TLDQPD SRAEQLNAA LNHMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWHL LNKNWSSVTVP TLIIGADLDTIAPVATHAKPFYN SLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRD GGEVCEYRSTCPF
60	MAANPYERGNPTDALLEARSGPFSVSEENVSR L S A S G F G G G T I Y Y P R E N S E N TYGAV AISPGYTGT QASIAWLGERIASHGFV VITIDTNT TLDQPD SRRARQLNA ALDYMINDSA VRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVATHAKPFYN SLPSSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRD GGEVCEYRSTCPF
61	MAANPYERGNPTDALLEARSGPFSVSEENVSR L S A S G F G G G T I Y Y P R E N S E N TYGAV AISPGYTGT QASIAWLGERIASHGFV VITIDTNT TLDQPD SRRARQLNA ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWH LNKNWSSVTVP TLIIGADLDTIAPVATHAKPFYN SLPSSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRD GGEVCEYRSTCPF
62	MAANPYERGNPTDALLEARSGPFSVSEENVSR L S A S G F G G G T I Y Y P R E N S E N TYGAV AISPGYTGT QASIAWLGERIASHGFV VITIDTNT TLDQPD SRRARQLNA ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVATHAKPFYN SLPSSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRD GGEVCEYRSTCPF
63	MAANPYERGNPTDALLEARSGPFSVSEENVSR L S A S G F G G G T I Y Y P R E N S E N TYGAV AISPGYTGT QASIAWLGERIASHGFV VITIDTNT TLDQPD SRRARQLNA ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWH LNKNWSSVTVP TLIIGACLDTIAPVATHAKPFYN SLPSSISKAYLELDGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRD GGEVCEYRSTCPF
64	MAANPYERGNPTDALLEARSGPFSVSEENVSR L S A S G F G G G T I Y Y P R E N S S N TYGAV AISPGYTGT EASIAWLGERIASHGFV VITIDTIT TLDQPD SRAEQLNAA LNHMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWHL LNKNWSSVTVP TLIIGACLDTIAPVATHAKPFYN SLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRD GGEVEEYRSTCPF
65	MAANPYERGNPTDALLEARSGPFSVSEENVSR L S A S G F G G G T I Y Y P R E N S E N TYGAV AISPGYTGT QASIAWLGERIASHGFV VITIDTNT TLDQPD SRRARQLNA ALDYMINDSA VRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWH LNKNWSSVRVPTLIIGACLDTIAPVATHAKPFYN SLPSSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRD GGEVEEYRSTCPF
66	MAANPYERGNPTDALLEARSGPFSVSEENVSR L S A S G F G G G T I Y Y P R E N S E N TYGAV AISPGYTGT QASIAWLGERIASHGFV VITIDTNT TLDQPD SRRARQLNA ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWH LNKNWSSVTVP TLIIGACLDTIAPVATHAKPFYN SLPSSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRD GGEVEEYRSTCPF
67	MAANPYERGNPTDALLEARSGPFSVSEENVSR L S A S G F G G G T I Y Y P R E N S E N TYGAV AISPGYTGT QASIAWLGERIASHGFV VITIDTNT TLDQPD SRRARQLNA ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWH LNKNWSSVRVPTLIIGACLDTIAPVATHAKPFYN SLPSSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRD GGEVEEYRSTCPF
68	MAANPYERGNPTDALLEARSGPFSVSEENVSR L S A S G F G G G T I Y Y P R E N S E N TYGAV AISPGYTGT QASIAWLGERIASHGFV VITIDTNT TLDQPD SRRARQLNA

	ALDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVRVPTLIIGARLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
69	MAANPYERGNPTDALLEARSGPFSVSEENVSRLSASGFGGGTIYYPRENSEN TYGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNA ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVTVPPTLIIGARLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
70	MAANPYERGNPTDALLEARSGPFSVSEENVSRLSASGFGGGTIYYPRENSEN TYGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNA ALNYMINRSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVRVPTLIIGARLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
71	AANPYERGNPTDALLEARSGPFSVSEERASRLSADGFGGGTIYYPRENSEN YGAVAIISPGYTGTQASMAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNA ALDHMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSNVRVPTLIIGADLDTIAPVLTHAKPFYNSLPTSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTGPF
72	AANPYERGNPTDALLEARSGPFSVSEERASRLSADGFGGGTIYYPRENSEN YGAVAIISPGYTGTQASMAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNA ALNHMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVLTHAKPFYNSLPTSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTGPF
73	AANPYERGNPTNALLEARSGPFSVSEENVSRLSADGFGGGTIYYPRENSEN YGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
74	AANPYERGNPTDALLEARSGPFSVSEENVSRLSADGFGGGTIYYPRENSEN YGAVVISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
75	AANPYERGNPTDALLEARSGPFSVSEERVSRLSADGFGGGTIYYPRENSEN YGAVAIISPGYTGTQASMAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNA ALDHMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVLTHAKPFYNSLPTSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLPPGPRDGGGEVCEYRSTGPF
76	MANPYERGNPTEALLEARSGPFSVSEERASRLGADGFGGGTIYYPRENSEN YGAVAIISPGYTGTQASVAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMLNDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKSWSNVQVPTLIIGADLDTIAPVLTHAEPFYNSIPTSTSKAYLELDGATHFA PNITNKTIGMYSVAWLKRFVDEDTRYTQFLCPGPRTGSDVEEYRSTCPF
77	AANPYERGNPTDALLEARSGPFSVSEETVSRLSASGFGGGTIYYPRENSEN YGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGDVCEYRSTCPF
78	AANPYERGNPTDALLEARSGPFSVSEENVSRLSADGFGGGTIYYPRENSEN YGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA

	LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKTWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVCEYRSTCPF
79	AANPYERGNPTDALLEARRGPFSVSEENVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVCEYRSTCPF
80	AANPYERGNPTDALLEARSGPFSVSEERASRLGADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASMAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNA ALDHMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVLTHAKPFYNSLPTSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLPPGPRTGGEVCEYRSTGPF
81	MANPYERGNPTDALLEARSGPFSVSEERASRLGADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASMAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNA ALDHMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSNVRVPTLIIGADLDTIAPVLTHAKPFYNSLPTSISKAYLELDGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLPPGPRTGGEVSEYRSTGPF
82	MANPYERGNPTDALLEARSGPFSVSEERASRLGADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASVAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVTVPPTLIIGADLDTIAPVLTHARPFYNSLPTSISKAYLELDGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVEEYRSTCPF
83	MANPYERGNPTEALLEARSGPFSVSEERASRLGADGFGGGGTIYYPRENSDNT YGAVAIISPGYTGTQASVAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMLNDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKSWSNVQVPTLIIGADLDTIAPVLTHAEPFYNSIPTSTSKAYLELDGATHFA PNITNKTIGMYSVAWLKRFVDEDTRYTQFLCPGPRTGSDVEEYRSTCPF
84	AANPYERGNPTDALLEARSGPFSVSEENVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASMAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNA ALDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVCEYRSTCPF
85	AANPYERGNPTDALLEARSGPFSVSEENVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRAKQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVCEYRSTCPF
86	AANPYERGNPTDALLEARSGPFSVSEENVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLGVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGEVCEYRSTCPF
87	MANPYERGNPTDALLEARSGPFSVSEERASRLGADGFGGGGTIYYPRENSDN TYGAVAIISPGYTGTQASMAWLGERIASHGFFVITIDTNTTLDQPDSRARQLN AALDHMINDSTVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPW HLNKNWSNVRVPTLIIGADLDTIAPVLTHAKPFYNSLPTSISKAYLELCGATHF APNIPNKTIGKYSVAWLKRFVDNDTRYTQFLPPGPRTGGEVCEYRSTGPF
88	AANPYERGNPTDALLEARSGPFSVSEERASRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASMAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNA

	ALDHMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVLTHAKPFYNSLPTSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLPPGPRDGGGEVCEYRSTGPF
89	MANPYERGNPTEALLEARSGPFSVSEERASRLGADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASVAWLGERIASHG FVVITIDTNTTLDQPDSRARQLNAA LDYMLNDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWH LNKSWSNVTVP TLIIGADLDTIAPVLTHAEPFYNSIPTSTSKAYLELDGATHFA PNITNKTIGMYSVAWLKRFVDEDTRYTQFLCPGPRTGSDVEEYRSTCPF
90	MANPYERGNPTEALLEARSGPFSVSEERASRLGADGFGGGGTIYYPRENSSNT YGAVAIISPGYTGTQASVAWLGERIASHG FVVITIDTNTTLDQPDSRARQLNAA LDYMLNDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWH LNKSWSNVQVPTLIIGADLDTIAPVLTHAEPFYNSIPTSTSKAYLELDGATHFA PNITNKTIGMYSVAWLKRFVDEDTRYTQFLCPGPRTGSDVEEYRSTCPF
91	AENPYERGPDPTESSIEASRGPFVAVSETSVSRLSVSGFGGGGTIYYPTSTSEGTFG AVAIISPGYTATQSSIAWLGPRLASQGFVVITIDTNSRYDQPDSRARQLLAALD YLTNSSSVRSRIDPSRLAVMGHSMGGGGTLQAAEDRPSLKAAIPLTPWHTDK NWSSVRVPTLIIGAENDTVAPVSSHAKPFYNSLPSSPEKAYLELNGASHFAPN SSNTTIKYSIAWLKRFVDDDTRYEQFLCPAPSTDSAISEYRSTCPY
92	AANPYERGNPTDALLEARSGPFSVSEENVSRLSASGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASMAWLGERIASHG FVVITIDTNTTLDQPDSRARQLNA ALDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGDVCEYRSTCPF
93	AANPYERGNPTDALLEARSGPFSVSEESVSRLSASGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIASHG FVVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGDVCEYRSTCPF
94	AANPYERGNPTDALLEARSGPFSVSEENVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIASHG FVVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSMIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
95	AANPYERGNPTDALLEARSGPFSVSEENVSRLSASGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIASHG FVVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWHL NKNWNSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGDVCEYRSTCPF
96	AANPYERGNPTDALLEARSGPFSVSEENVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIASHG FVVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGDVCEYRSTCPF
97	AANPYERGNPTNALLEARSGPFSVSEENVSRLSASGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIASHG FVVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPD LKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGDVCEYRSTCPF
98	AANPYERGNPTDALLEARSGPFSVSEENVSRLSASGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIASHG FVVITIDTNTTLDQPDSRARQLNAA

	LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGGTHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGDVCEYRSTCPF
99	QANPYERGPNTDALLEARSGPFSVSEENVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
100	AANPYERGPDPTESSLEASSGPFAVSETSVSRLSVSGFGGGGTIYYPTSTSSGT GAVAIISPGYTATQSSIAWLGPRLASHGFFVITIDTNSRFDQPDSRARQLLAAL NYLVNSSSVRSRIDSSRLAVMGHSMGGGGTLRAAEDRPSLKAAIPLTPWHTD KNWSSVTVPTLIIGAENDTVAPVSSHAKPFYNSLPSSTPKAYLELNGASHFAP NSSNTTIGKYSIAWLKRFVDNDTRYSQFLCPAPSDDSAISEYRSTCPY
101	MANPYERGPNTDALLEARSGPFSVSEERASRLGADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASVAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVLTHARPFYNSLPTSISKAYLELDGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGVEVEYRSTCPF
102	AANPYERGPNTDALLEARSGPFSVSEENVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWNSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
103	AANPYERGPNTDALLEARSGPFSVSEENVSRLSADGFGGGGTIYYPRENSTNT YGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
104	AANPYERGPNTDALLEARSGPFSVSEERVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASMAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNA ALNHMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVLTHAKPFYNSLPTSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLPPGPRDGGGEVCEYRSTGPF
105	AENPYERGPDPTESSIEALRGPFAVSEESVSRLSVSGFGGGGTIYYPTDSTSEGT GAVAIISPGYTGTQSSMAWLGPRIASQGFVFTIDTNTTYDQPDSRARQLQAA LDYLVEDSSVRDRIDPNRLGVMGHSMGGGGTLRAAEDRPDLKAAIPLTPWH LNKNWSSVRVPTLIIGAENDTIAPVRTHAEPFYESLPSSLDKAYLELDGASHFA PNISNTTIAKYSISWLKRFVDDDTRYEQFLCPPPRTGSEISEYRSTCPF
106	AANPYERGPNPTEALLEARSGPFSVSEERASRLGADGFGGGGTIYYPRENSDNT YGAVAIISPGYTGTQASVAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVLTHAEPFYNSLPTSISKAYLELDGATHFAP NITNKTIGKYSVAWLKRFVDEDTRYTQFLCPGPRTGSDVEEYRSTCPF
107	AANPYERGPNTDALLEARSGPFSVSEERASRLGADGFGGGGTIYYPRENSDNT YGAVAIISPGYTGTQASVAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVLTHAKPFYNSLPTSISKAYLELDGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGVEVEYRSTCPF
108	AANPYERGPNTDALLEARSGPFSVSEERVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIASHGFFVITIDTNTTLDQPDSRARQLNAA

	LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
109	AANPYERGNPTDALLEARSGPFSVSEENVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIAHGFVVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGGTHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
110	AANPYERGNPTDALLEARSGPFSVSEENVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIAHGFVVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGREVCEYRSTCPF
111	AANPYERGNPTDALLEARSGPFSVSEENVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIAHGFVVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFTP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
112	AANPYERGNPTDALLEARSGPFSVSEERVSRLGADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASMAWLGERIAHGFVVITIDTNTTLDQPDSRARQLNA ALDHMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKNWSSVRVPTLIIGADLDTIAPVLTHAKPFYNSLPTSISKAYLELCGATHFA PNIPNKIIGKYSVAWLKRFVDNDTRYTQFLPPGPRDGGGEVCEYRSTGPF
113	AANPYERGPDPTESSLEASSGPFSVSETSVSRLSVSGFGGGGTIYYPTDTSSGT YGAVAISPGYTATQSSIAWLGPRLASHGFVVITIDTNSTFDQPDSRARQLMAAL DYLVNSSSVRSRIDSSRLAVMGHSMGGGGTLRAEDNPSLKAAIPLTPWHTD KNWSSVRVPTLIIGAENDTVAPVSSHAKPFYNSLPSSTPKAYLELNGASHFAP NSSNTTIGKYSVSWLKRFVDNDTRYSQFLCPAPHDDSAISEYRSTCPY
114	AANPYERGNPTEALLEARSGPFSVSEERASRLGADGFGGGGTIYYPRENSDNT YGAVAIISPGYTGTQASVAWLGERIAHGFVVITIDTNTTLDQPDSRARQLNAA LDYMLNDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWH LNKSWSNVQVPTLIIGADLDTIAPVLTHAEFYNSIPTSTSKAYLELDGATHFA PNITNKTIGMYSVAWLKRFVDEDTRYTQFLCPGPRTGSDVEEYRSTCPF
115	AANPYERGNPTDALLEARSGPFSVSEESVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIAHGFVVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
116	AANPYERGNPTDALLEARSGPFSVSEENVSRLSASGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIAHGFVVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKTWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGDVCEYRSTCPF
117	AANPYERGNPTDALLEARSGPFTVSEENVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIAHGFVVITIDTNTTLDQPDSRARQLNAA LDYMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
118	AANPYERGNPTDALLEARSGPFSVSEENVSRLSADGFGGGGTIYYPRENSENT YGAVAIISPGYTGTQASIAWLGERIAHGFVVITIDTNTTLDQPDSRARQLNAA

	LDHMINDSAVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPWHL NKNWSSVRVPTLIIGADLDTIAPVATHAKPFYNSLPSSISKAYLELCGATHFAP NIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPGPRDGGGEVCEYRSTCPF
119	GPXPTXXXXEAXXGPFXXXXXXXXXXXXXXXXXGFGGGTIYYPXXXSXXXXGAV XI SPGXTXXXSXAWLGXRASXGFVVXTIDTXXXXDQPXXRXXQLXAALXX XXXXSXVRXX XDXXRLXVXGHSMGGGGTLXXAXXXPXLXAAXPLXXWXXXKXXXXVXVP TXXIGXXXDTX APVXXHXXPFYXSXPXXXXKAYXELXXXXHXXPXXXNXXXXXYXXXWKK RFXDXDTRYXX FLXXXPXXXXXXXXXYYRXXXPX 其中X可為任何胺基酸。

表2：三種例示性DOCT酶多肽之效率

SEQ ID NO	MOCT濃度(峰面積)	TPA濃度(峰面積)	累積MOCT/TPA (峰面積)
6	33.33000946	157.8963776	191.226387
7	128.8890686	184.8075409	313.6966095
8	71.81786346	180.6810913	252.4989548

表3：三種例示性MOCT酶多肽之效率

SEQ ID NO	TPA濃度(峰面積)
9	7304.587011
10	7310.312197
11	6510.802634

【0159】 本文所引用之每一專利、專利申請案及公開案之揭示內容特此以全文引用之方式併入本文中。

【0160】 本文中任何參考文獻之引用不應解釋為承認此類參考文獻可作為「先前技術」用於本申請案。

【0161】 在整個本說明書中，目標為描述本發明之較佳實施例，而不將本發明限制於任一個實施例或特定特徵集合。熟習此項技術者因此將瞭解，根據本發明，可在不脫離本發明之範疇的情況下在特定實施例中進行各種修改及改變。所有此類修改及改變意欲包括在所附申請專利範圍之範疇內。

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【發明申請專利範圍】

【請求項1】

一種具有酯酶活性之多肽，其中該酯酶活性能夠：

- a. 將對苯二甲酸單酯轉化成對苯二甲酸及醇；
- b. 將對苯二甲酸二酯轉化成對苯二甲酸單酯及醇；或
- c. 將對苯二甲酸二酯轉化成對苯二甲酸及醇；

其中該多肽包含選自由以下組成之群的胺基酸序列：

i. SEQ ID NO:2之胺基酸5-261或與其具有至少85%序列一致性之胺基酸序列；

ii. SEQ ID NO: 3之胺基酸5-261或與其具有至少77%序列一致性之胺基酸序列；

iii. SEQ ID NO: 4之胺基酸5-261或與其具有至少75%序列一致性之胺基酸序列；

iv. SEQ ID NO: 5之胺基酸5-261或與其具有至少95%序列一致性之胺基酸序列；及

v. SEQ ID NO: 6之胺基酸5-261或與其具有至少96%序列一致性之胺基酸序列，

其中該多肽不為SEQ ID NO: 1或SEQ ID NO: 12。

【請求項2】

如請求項1之多肽，其中該多肽包含SEQ ID NO: 6之胺基酸5-261之胺基酸序列或與其具有至少96%序列一致性之胺基酸序列。

【請求項3】

如請求項2之多肽，其中該多肽包含SEQ ID NO: 6之胺基酸5-261之

胺基酸序列。

【請求項4】

如請求項1之多肽，其中該多肽包含SEQ ID NO: 7之胺基酸5-261之胺基酸序列或與其具有至少97%一致性之胺基酸序列。

【請求項5】

如請求項4之多肽，其中該多肽包含SEQ ID NO: 7之胺基酸5-261之胺基酸序列。

【請求項6】

如請求項1之多肽，其中該多肽包含SEQ ID NO: 8之胺基酸5-261之胺基酸序列或與其具有至少96%一致性之胺基酸序列。

【請求項7】

如請求項6之多肽，其中該多肽包含SEQ ID NO: 8之胺基酸5-261之胺基酸序列。

【請求項8】

如請求項1之多肽，其中該多肽包含SEQ ID NO: 9之胺基酸5-261之胺基酸序列或與其具有至少97%一致性之胺基酸序列。

【請求項9】

如請求項8之多肽，其中該多肽包含SEQ ID NO: 9之胺基酸5-261之胺基酸序列。

【請求項10】

如請求項1之多肽，其中該多肽包含SEQ ID NO: 10之胺基酸5-261之胺基酸序列或與其具有至少98%一致性之胺基酸序列。

【請求項11】

如請求項10之多肽，其中該多肽包含SEQ ID NO: 10之胺基酸5-261之胺基酸序列。

【請求項12】

如請求項1之多肽，其中該多肽包含SEQ ID NO: 11之胺基酸5-261之胺基酸序列或與其具有至少98%一致性之胺基酸序列。

【請求項13】

如請求項12之多肽，其中該多肽包含SEQ ID NO: 11之胺基酸5-261之胺基酸序列。

【請求項14】

如請求項1至13中任一項之多肽，其中該多肽包含SEQ ID NO: 119之胺基酸序列。

【請求項15】

一種酵素水解作為PET降解之副產物產生的對苯二甲酸單酯及/或對苯二甲酸二酯之方法，

其中

a)該對苯二甲酸單酯為對苯二甲酸單- C_1 - C_{10} 烷基酯，視情況經苯甲基取代，且其中該對苯二甲酸單酯不為對苯二甲酸單-(2-羥乙酯)(MHET)；

b)該對苯二甲酸二酯為對苯二甲酸二- C_1 - C_{10} 烷基酯，視情況經苯甲基取代；

該方法包含使該對苯二甲酸單酯及/或對苯二甲酸二酯暴露於具有酯酶活性之多肽，在足以使該多肽能夠轉化以下之條件下：

i. 將該對苯二甲酸單酯轉化成對苯二甲酸及醇；

- ii. 將該對苯二甲酸二酯轉化成對苯二甲酸單酯及醇；或
- iii. 將該對苯二甲酸二酯轉化成對苯二甲酸及醇。

【請求項16】

如請求項15之方法，其中該多肽包含SEQ ID NO: 1之胺基酸5-261之胺基酸序列或與其具有至少70%序列一致性之胺基酸序列。

【請求項17】

如請求項15或請求項16之方法，其中該對苯二甲酸單酯為對苯二甲酸單-C₆-C₁₀烷基酯，視情況經苯甲基取代。

【請求項18】

如請求項17之方法，其中該對苯二甲酸單酯係選自由以下組成之群：對苯二甲酸單苯甲酯(MBZT)、對苯二甲酸單己酯、對苯二甲酸單庚酯(MHPT)及對苯二甲酸單辛酯(MOCT)。

【請求項19】

如請求項18之方法，其中該對苯二甲酸單酯為MBZT或MOCT。

【請求項20】

如請求項17至19中任一項之方法，其中該多肽包含SEQ ID NO: 9-11中任一者之胺基酸5-261之胺基酸序列或與前述任一者具有至少70%序列一致性之胺基酸序列。

【請求項21】

如請求項17至19中任一項之方法，其中該多肽包含SEQ ID NO: 119之胺基酸序列。

【請求項22】

如請求項15或請求項16之方法，其中該對苯二甲酸二酯為對苯二甲

酸二-C₆-C₁₀烷基酯，視情況經苯甲基取代。

【請求項23】

如請求項22之方法，其中該對苯二甲酸二酯係選自由以下組成之群：對苯二甲酸二苯甲酯(DBZT)、對苯二甲酸二己酯(DHXT)、對苯二甲酸二庚酯(DHPT)及對苯二甲酸二辛酯(DOCT)。

【請求項24】

如請求項23之方法，其中該對苯二甲酸二酯為DBZT或DOCT。

【請求項25】

如請求項22至24中任一項之方法，其中該多肽包含SEQ ID NO: 6-8中任一者之胺基酸5-261之胺基酸序列或與前述任一者具有至少70%序列一致性之胺基酸序列。

【請求項26】

如請求項15至25中任一項之方法，其中該對苯二甲酸單酯及/或對苯二甲酸二酯係藉由包含以下之製程產生：

- a. 使PET暴露於氫氧化鈉，及/或
- b. 使該PET與酯酶接觸。

【請求項27】

如請求項26之方法，其中對苯二甲酸單酯及/或對苯二甲酸二酯係藉由包含以下之製程產生：

- c. 使該PET經歷利用C₁-C₁₀單醇之鹼催化之轉酯化；及/或
- d. 使該PET與酯酶接觸。

【請求項28】

如請求項27之方法，其中該C₁-C₁₀單醇為C₆-C₁₀單醇。

【請求項29】

如請求項28之方法，其中該C₆-C₁₀單醇為苯甲醇、辛醇或庚醇。

【請求項30】

如請求項29之方法，其中該C₆-C₁₀單醇為1-辛醇。

【請求項31】

如請求項15至30中任一項之方法，其進一步包含回收該對苯二甲酸及/或該醇。

【請求項32】

一種組合物，其包含藉由如請求項31之方法回收的該對苯二甲酸及/或醇。

【請求項33】

一種組合物，其包含如請求項1至14中任一項之多肽。

【請求項34】

一種多核苷酸，其包含編碼如請求項1至14中任一項之多肽之核酸序列。

【請求項35】

一種表現卡匣或載體，其包含如請求項34之多核苷酸。

【請求項36】

一種宿主細胞，其包含：

- a) 如請求項1至14中任一項之多肽，
- b) 如請求項34之多核苷酸，或
- c) 如請求項35之表現卡匣或載體。

【請求項37】

一種宿主細胞，其經基因修飾以表現如請求項1至14中任一項之多肽。

【請求項38】

一種產生具有酯酶活性之多肽之方法，該方法包含

a)提供如請求項34之多核苷酸；

b)在足以允許宿主細胞產生該多肽之條件下在該宿主細胞中表現該多核苷酸；及

c)收集藉由(b)中之宿主細胞產生的多肽。

【請求項39】

一種使包含PET之塑膠產品再循環之方法，該方法包含

a)藉由鹼催化之轉酯化來降解該PET以產生對苯二甲酸單酯及/或對苯二甲酸二酯，及

b)使該對苯二甲酸單酯及/或對苯二甲酸二酯與如請求項1至13中任一項之多肽、如請求項33之組合物或如請求項36或請求項37之宿主細胞在足以使得該多肽能夠轉化以下條件下接觸：

i. 將該對苯二甲酸單酯轉化成對苯二甲酸及醇；

ii. 將該對苯二甲酸二酯轉化成對苯二甲酸單酯及醇；或

iii. 將該對苯二甲酸二酯轉化成對苯二甲酸及醇。

【請求項40】

如請求項39之方法，其中降解該PET進一步包含使該PET同時或依序暴露於PET酶及/或具有PET酶活性之角質酶(cutinase)。

【請求項41】

如請求項33或請求項34之方法，其中步驟b)進一步包含使該對苯二

甲酸單酯及/或該對苯二甲酸二酯同時或依序暴露於MHET酶或具有MHET酶活性之酵素。

【請求項42】

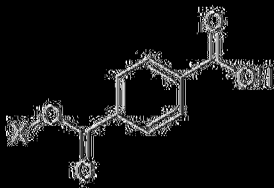
一種具有酯酶活性之多肽，其中該酯酶活性能夠

- a. 將對苯二甲酸單酯轉化成對苯二甲酸及醇；
- b. 將對苯二甲酸二酯轉化成對苯二甲酸單酯及醇；或
- c. 將對苯二甲酸二酯轉化成對苯二甲酸及醇；

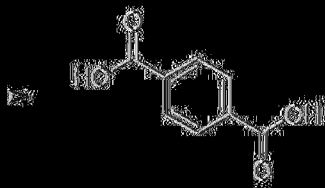
其中該多肽包含SEQ ID NO: 119之胺基酸序列。

|(發明圖式)|

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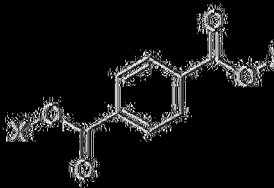
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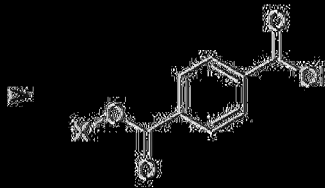
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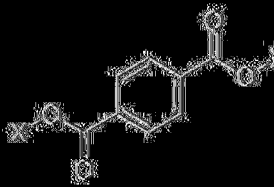
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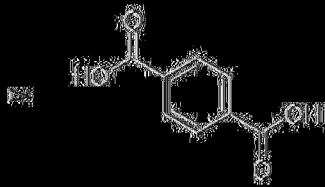
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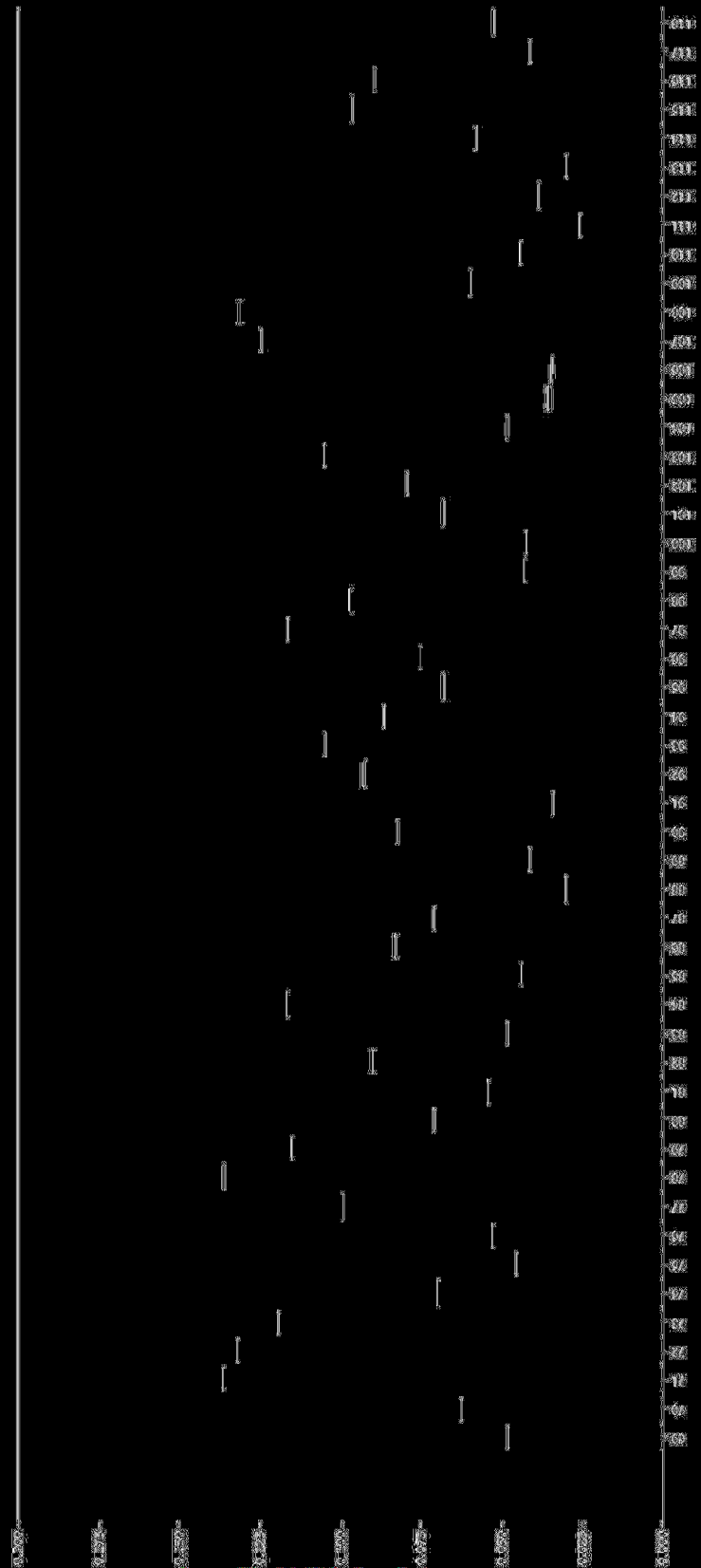
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