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論文名稱(英)	The analysis and application of endogenous IS1405s of Ralstonia solanacearum
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摘要(中)	IS1405 和 IS5 family 中的 11 個 IS 成員，使用 maximum parsimony analysis 建構親緣關係圖，結果顯示在 IS5 family 的六個 subgroups：IS1031、IS427、ISL2、ISH1、IS903 和 IS5 subgroups 中，IS1405 屬於 IS5 subgroup。同時發現屬於 IS5 subgroup 的 IS 成員中，來自相同菌種的 IS，在親緣關係圖中位於較近的分支，顯示 IS5 subgroup 中的 IS 成員親緣關

	係和菌種來源的分類地位相同。同屬於 IS5 subgroup 的 IS 成員：IS1405、IS1384、IS1051、IS1479a、ISPSMC 和 IS1646 的核酸序列比對中，在 coding region 相同度由 51-60%不等，5"和 3" non-coding region 中相同度降至 22-30%不等，顯示 5"和 3" non-coding region 核酸序列的變異大於 coding region。利用 IS1405 的 5"和 3" non-coding region 和其他 IS5 subgroup 成員差異大的核酸序列，設計 polymerase chain reaction (PCR)反應的引子對，利用此引子對對來自七個不同寄主的 26 株青枯病菌菌株進行 PCR 擴增反應，皆有預期的 1.1 kb 片段產生，靈敏度達 70 cfu/ml 和 1.6×10-3ng/ml。但對其他植物病原菌，如 Erwinia 和 Xanthomonas 等進行 PCR 反應，則無任何預期的片段產生，因此利用 IS1405 序列所設計的此組引子對，對青枯病菌具有專一性的檢測功用，並證實 IS1405 只存在於青枯病菌中，所以 IS1405 是一個 species-specific 的插入序列。 自青枯病菌基因組中，以 inverted PCR 和直接選殖含有 IS1405 的 EcoRI 片段這兩種方式，取得七個含有 IS1405 的核酸片段，分析這七個 IS1405 在青枯病菌基因組中，座落位置兩旁的核酸序列。在目標位置（target site）分析方面，有五個 IS1405 的 target site 為 CTAG，另有兩個為 CTAA，綜合以上可知 IS1405 具有高度專一性的 target site 選擇，序列為 CTAR (R = A 或 G)；其中有五個 IS1405 是以和 target site 相同的方向插入，另外兩個則以和 target site 相反的方向插入，顯示 IS1405 在辨認位置後，可用不同的方向插入目標位置中；在 IS1405 插入位置兩旁序列比對中，排列在 IS1405 左旁邊為 5"-CCT(2)T(3)CAC(3)CGC(3)T(4)T(6)G(13) TAG-3"的保守序列；在 IS1405 右旁邊的序列，則為 5"-AG(3)T(14)T(2)ATA(13)T-3"的保守序列。另以 IS1405 兩端的 inverted repeat (IR)序列設計引子，藉由 PCR 方式對青枯病菌基因組中含有 IS1405 的不同 EcoRI 片段，擴增出七個 isoIS1405 核酸序列，加上之前選殖的三個 isoIS1405 核酸序列，共十個進行分析。這十個 isoIS1405 核酸序列，有五個來自青枯病菌菌株 PS-CTW31 的不同片段，三個來自青枯病菌菌株 PS68，另外兩個分別來自於青枯病菌菌株 PS96 和 PS95。在序列長度分析，這十個 isoIS1405 的核酸序列皆為 1,174 bp，在序列 67-1,030 bp 處可轉譯出長度為 321 個胺基酸的蛋白質序列，此段胺基酸中包含有跳躍酵素特有的序列。在核酸序列比對中，十個 isoIS1405 相同度由 95%-99%不等，並可依據核酸序列的差異，將十個 isoIS1405 區分為一、二兩類，在同一類中核酸序列相同度為 98-99%，在不同類間核酸序列相同度為 95-97%不等；第一類的 isoIS1405 序列在 29 個地方具有相同，但和第二類的 isoIS1405 序列不同的核酸序列，也就是這兩類的 isoIS1405 核酸序列，是利用這 29 個核酸序列的差異作區別。在基因演化的過程中，29 個核酸序列同時突變的機率接近於零，因此推測這兩類的 isoIS1405 的形成是個別獨立的，而不是經由隨機突變形成的。同時，在將 isoIS1405 的 PCR 擴增片段以 AvaI 處理後跑電泳的實驗結果中，也顯示 isoIS1405 可區分為 AvaI-1 和 AvaI-2 兩類，在 AvaI-1 中包含有和第一類 isoIS1405 相同的成員；AvaI-2 中包含有和第二類相同的 isoIS1405 成員，因此使用 PCR-RFLP 的方式，可將 isoIS1405 在核酸序列的變異情形快速的呈現出來。在 isoIS1405 的分佈方面，青枯病菌菌株 PS-CTW9 和 PS-ES10 中只含有屬於 AvaI-1 類的 isoIS1405，其他青枯病菌菌株如 PS68、PS-CTW25 等中，則同時存在有這兩類不同的 isoIS1405。

	以 IS1405 為探針，對來自七個不同寄主的 26 株青枯病菌菌株基因組，以 EcoRI 處理後進行南方氏雜合反應。依產生雜合片段的數目，可將青枯病菌區分成 A、B 兩大 group，A group 中含有七個以內的雜合片段；B group 中則有十二個以上的雜合片段，每一個 group 再依雜合型式的差異區分出多個 subgroups (A1-A5 和 B1-B7)。在 A1 subgroup 的菌株中，共同擁有 1.4 kb 的核酸片段，以 PCR 方式擴增出屬於 A1 subgroup 的青枯病菌菌株 PS68 的 1.4 kb 片段，命名為 IS1405BF，再以此片段 3"端距離 IS1405 插入位置 55 個 bp 處，設計引子 PSIS-RA1，此引子和 PSIS-F 共同使用，對青枯病菌菌株進行 PCR 擴增反應，只有屬於 A1 subgroup 的青枯病菌能擴增出 1,181 bp 的預期片段。同時，選殖出青枯病菌菌株 PS-CTW31 和其他 B1 subgroup 中的菌株，都共同擁有的 2 kb EcoRI 核酸片段，命名為 IS1405 FF，並以此片段 3"端距離 IS1405 插入位置 130 個 bp 處的序列設計引子 PSIS-RB1，和 PSIS-F 共同使用，對青枯病菌菌株進行 PCR 擴增反應，只有屬於 B1 subgroup 的青枯病菌能擴增出 1,256 bp 的預期片段，因此使用 PCR 方式可快速區分出屬於 A1 和 B1 subgroups 的青枯病菌菌株。以 IS1405 5"和 3" non-coding region 設計的引子對 PSIS-F+PSIS-R，可專一性的檢測青枯病菌，而以 IS1405 在基因組中座落位置兩旁的片段 IS1405BF 和 IS1405FF 設計的引子對，可利用 PCR 方式快速區分出 A1 和 B1 兩大 subgroups，所以利用 IS1405 對青枯病菌進行檢測和分類是實用且快速的方法。
摘要 (英)	IS1405 of Ralstonia solanacearum belongs to the IS5 subgroup of IS5 family based on phylogenetic tree analysis of nucleotide sequences using the maximum parsimony analysis and comparison of terminal nucleotide sequences. Further comparison of IS1405 with IS5 subgroup members, such as IS1384, IS1051, IS1479a, ISPSMC and IS1646, revealed that the nucleotide sequence of coding region (encodes transposase) shared identity from 51-60%, but the identity of 5" and 3" non-coding region ranged from 22 to 30%. Oligonucleotide primers (PSIS-F + PSIS-R) specific to IS1405 were designed for polymerase chain reaction (PCR) based on the nucleotide sequence of 5" and 3"non-coding regions. The primers amplified a 1.1-kb PCR product only from R. solanacearum but not from other plant pathogenic bacteria, such as Erwinia and Xanthomonas spp. Accordingly, oligonucleotide primers (PSIS-F + PSIS-R) are specific not only to IS1405 but also to R. solanacearum. The analysis of flanking sequences of six endogenous isoIS1405s of R. solanacearum revealed that IS1405 has a preferred target site, CTAR(R=A or G), and can transpose into this target site at two different orientations. Further analysis of the 50-bp flanking sequences of endogenous IS1405s showed that there are consensus sequence of 5"-CCT(2)T(3)CAC(3)CGC(3)T(4)T(6)G(13) TAG-3" at the left flanking sequence and that of 5"-AG(3)T(14)T(2)ATA(13)T-3" at the right. In addition, nucleotide sequence of ten endogenous isoIS1405s isolated from 3 different strains of R. solanacearum were analyzed. The results indicated that all isoIS1405s are 1,174 bp nucleotide long and consisted of one open reading frame encoding a protein of 321 amino acids. The nucleotide sequences were highly conserved and shared 95-99% identity. Treatment of these IsoIS1405s with AvaI showed two different restriction

	fragment length polymorphism (RFLP) patterns, AvaI-1 and AvaI-2 types. Two types of IS1405s coexist in most strains of R. solanacearum. An internal fragment of IS1405 was used as a probe to hybridize EcoRI-treated total DNA of R. solanacearum. The results revealed that strains of R. solanacearum in Taiwan had an extensive genetic variability. The strains were classified into two distinct groups based on number of hybridized DNA fragments. The number of hybridized bands of group A varied from 4 to 7, and that of group B was more than 12. The strains in each group were further divided into subgroups (A1-A5 and B1-B7) according to hybridization patterns. Strains of A1 subgroup had a common 1.4-kb hybridized DNA fragment. The flanking sequence of IS1405 in 1.4-kb DNA fragment was determined and used to design a PCR primer, PSIS-RA1. PCR reaction using PSIS-RA1 and PSIS-F as primers amplified a specific PCR product only from the strains of A1 subgroup but not from those of other subgroups. The same strategy was also applied to the strains of B1 subgroup, which contained a common 2-kb hybridized DNA fragment. PSIS-RB1 was designed based on the flanking sequence of IS1405 in the fragment. A specific PCR product was amplified only from strains of B1 subgroup not from those of other subgroups using PSIS-RB1 and PSIS-F as primers.
論文目次	中文摘要 英文摘要 前言 材料與方法 菌種及培養條件 IS1405 和 IS5 family 成員的比較 分析青枯病菌菌株中 IS1405 座落位置兩旁的核酸序列 序列分析 IS1405 在青枯病菌 race 1 各菌株中存在情形 isoIS1405 的選殖 isoIS1405 的定序分析 PCR-RFLP 的方式 實驗結果 IS1405 與 IS5 family 成員間的序列比對分析 IS5 supgroup 間的核酸序列比對 利用 PCR 方式檢測青枯病菌 IS1405 在青枯病菌基因組中插入位置兩旁序列的分析 （1）選殖青枯病菌基因組中帶有 IS1405 之 DNA 片段 （2）含有 IS1405 之青枯病菌 DNA 片段分析 （3）IS1405 插入兩旁序列並列分析 isoIS1405 的選殖及序列比對分析 （1）isoIS1405 序列的選殖 （2）isoIS1405 序列分析 （3）isoIS1405 利用 PCR-RFLP 分類 以 IS1405 在青枯病菌 race1 各菌株中存在的情況 以 IS1405 座落位置區分青枯病菌 subgroup 討論 參考文獻 圖表 英文縮寫表
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