副結核分支桿菌基因多型性之研究

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摘要

副結核分支桿菌*Mycobacterium avium* subspecies *paratuberculosis* (MAP)引起副結核病或稱約內氏病，為一種肉芽腫性腸炎，本病感染的主要來源是被污染的飼料。臨床症狀主要為慢性漸進性的消耗及下痢，病灶發生於小腸壁與腸繫淋巴，會造成蛋白質流失及蛋白質吸收不良症。目前應用於MAP的基因多型性分類方法包括IS*900*及IS*1311*的RFLP (restriction fragment length polymorphism)、SSR (short sequence repeats)、MIRU (Mycobacterial interspersed repetitive units)、VNTR (variable number tandem repeat)、RAPD (randomly amplified polymorphic DNA)以及PFGE (pulse field gel electrophoresis)等。於本次研究中以兩種分型方法針對23株MAP的多型性進行初步的評估，所使用的試驗方法包括以SSR方法分析序列的多型性(包括一個、兩個及三個核苷酸的重覆)及9種VNTR方法分析片段大小的多型性。結果23株MAP於SSR方法分析G1、G2、GCG、GGT及TGC的片段重複下分別顯示出4、3、2、2、3種不同模式。而9種不同VNTR PCR反應分析下，其中2種可達分型的目的。其中最大族群依照SSR1-SSR2-SSR6-SSR8-SSR9-VNTR3 -VNTR4排列之模式為(≧14)-(≧11)-5-5-5-I-I，佔分析菌株比例34.8%(8/23)。由此結果可知利用SSR及VNTR技術計算重複位點的方法來分析MAP菌株具有良好的分型效果(*D*=0.86)。

Study on the genomic polymorphism of *Mycobacterium avium* subsp. *paratuberculosis*

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Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes paratuberculosis or Johne's disease, an intestinal granulomatous infection. The disease spreads by ingestion of MAP from the contaminated environment. Lesions occur in the small intestine and the draining mesenteric lymph nodes which are responsible for a protein leak and a protein malabsorption syndrome. The genotyping methods applied to MAP include IS*900*- or IS*1311*-RFLP (restriction fragment length polymorphism), SSR (short sequence repeats), MIRU (Mycobacterial interspersed repetitive units), VNTR (variable number tandem repeat), RAPD (randomly amplified polymorphic DNA), and PFGE (pulse field gel electrophoresis). Our preliminary study is to evaluate the efficiency of two methods to determine the molecular diversity of 23 MAP strains. The applied methods included the analysis of sequence polymorphism of the mono-, di-, and trinucleotide sequences of SSR and the determination of size polymorphism of 9 different VNTR. Sequence analysis of SSR of 23 isolates showed 4, 3, 2, 2, and 3 alleles of G1, G2, GCG, GGT and TGC repeats. And out of 9 VNTR PCR differentiation methods, only two methods could be recommended for typing purpose. The profile (≧14)-(≧11)-5-5-5-I-I of the combination systems SSR1-SSR2-SSR6-SSR8-SSR9-VNTR3-VNTR4 dominates among the examined isolates and was detected in 34.8%(8/23) of the isolates. The use of certain repetitive loci of SSR and VNTR techniques showed great potential for the characterization of MAP isolates (D=0.86).