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16S～23S rDNA间区在链球菌和流感嗜血杆菌分类中的应用

Identification of Streptococcus Species and Haemophilus influenzae by Direct Sequencing of PCR Products from 16S~23S rRNA Itergenic Sacer Rgions

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摘要　利用16S~23S rDNA间区（intergenic spacer regions,ISR）在不同细菌中拷贝数、碱基排列、序列长度及所含tRNA基因种类和数目的差异，对15株链球菌和流感嗜血杆菌进行属、种、型和株系的分类鉴定。在16S rDNA的3′端和23S rDNA的5′端的保守区中合成引物，PCR扩增16S~23S rDNA ISR序列，对多态片段切胶纯化直接测序。在GenBank上查找对应细菌的ISR序列。用DNAMAN软件进行系统进化分析。链球菌属为单拷贝16S~23Sr RNA ISR、有一个tRNAAla基因编码区、分子大小在269~446bp之间，序列分成4个保守区和4个可变区，可变区碱基排列方式和数目的不同是种分类的依据。7株链球菌的同源率在78%~88%。同种异株的差异反映在碱基的插入和缺失上。流感嗜血杆菌各生物型均为2个拷贝的ISR，小片段为514~519bp，编码1个tRNAGlu基因，有3个狭窄可变区。大片段富含A T碱基，在I、II和IV型中分别是868、848和856bp，编码一个tRNAIle基因和一个tRNAAla基因。不同生物型小分子ISR与标准菌株比较，同源性在97.3%~99.6 %之间。 ISR作为细菌分类的目的基因具有属、种、型和株特异性与灵敏性。简单的基因分离分析技术为认识病原微生物提供了更多的机会。

Abstract:To facilitate species level identification of bacteria without the requirement of presumptive identification,the paper describes a rapid identification method of bacteria by amplification and direct sequencing 16S~23S rDNA intergenic spacer regions (ISR) of the pathogens which cause the upper respiratory tract infective disease by Streptococcus and Haemophilus.Three pairs of primer targeting conserved sequences flanking the 3′ end of 16S and the 5′ end of 23S rRNA were used to amplify 16S~23S rRNA ISR of 7 streptococcus strains and 8 Haemophilus strains.The PCR products were separated by 1% agarose gel electrophoresis and the polymorphisms fragments were purified with the Wizard PCR Min-Prep Kit (Promega) and Protocol-SK131(Sangon).The nucleotide sequences of ISR inserts were determined by using the XEQTM DTCS Kit——Terminator Cycle Sequencing and a CEQTM 2000XL DNA Analysis system (Backman Coulter) automatic DAN sequencer.Then those sequences were compared with known seqnences on the GenBank.The alignment of nucleotide sequence,evolutionary distances and phylogenetic tress were analyzed by software DANMAN version 4.0.The PCR products were showed polymorphism patterns with agarose gel.One band was contained in streptococcus genus.The significant variation was found among the spacer sequences of different species in Streptococcus with the lengths of the spacer varying from 269 to 446bp.All the ISR of the streptococcal species had a tRNA Ala gene in the spacer and the sequence identities varied from 78 to 88% within genera.It was found that some spacer sequence blocks were highly conserved between operons of a genome,whereas the presence of others was variable,three regions showed significant spatial variation.Most of the differences between the sequences came from several bases insertions/deletions and substitutions.There are two major bands in the Haemophilus biotypes(515 and 884bp),the small ISR amplicon contained one tDNA coding for tRNAGlu.In contrast to the large one contained two tRNA genes coding for tRANAla and tRNAIle.Two regions of repeating motifs with only A or T were present in higher copy numbers between tRANAla and tRNAIle.The phylogenetic trees varied from 97.5 to 98.8%.The PCR and direct sequencing of 16S~23S rRAN ISR were successful in the pathogen species identification.

关键词　16S~23Sr RNA间区　链球菌　流感嗜血杆菌　分类　Key words　16S~23S rDNA inergenic spacer regions　Streptococcus　H.influenzae　identification

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