H302 Rapid diagnosis and typing of Enteroviruses as causative agents of Aseptic meningitis using PCR-RFLP.

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Aseptic menigitis, an acute inflammation of the meningitis, is a common illness during childhood. Virus is the most important cause of aseptic meningitis. Especially enterovirus causes approximately above 85% of all causes of aseptic meningitis. Current diagnostic techniques and serotyping procedures like neutralization test for the detection and typing of enteroviruses from the acute patients take too laborious time, and are not sensitive to distinguish several specific types. This study was so focused on the polymerase chain reaction(PCR) diagnostic procedures followed by restriction fragment length polymorphism(RFLP) of these PCR products for the rapid diagnosis and serotyping of the causative enteroviruses. seven kinds of enteroviruses, coxakievirus B1, B3, B5, B6, Echovirus 7, 9, and 30 which have been isolated and typed by RIVM serumpools in Korea since 1993, were analyzed by PCR-directed sequencing and RFLP test using restriction enzyme. With the primers like Ent1F, Ent2F, and EntR, which are flanking the 5′ noncoding region(5′NCR) of the enterovirus genome, we could get 436bp, 152bp of PCR products, and these products were sequenced by PCR-directed sequencing with the same primers. According to the sequence analysis, several different restriction enzyme recognition sites such as Nci I, Aos I, SfaN I, and Esp I were found in the 5′NCR which have been reported as the highly conserved regions among different subtypes, and fragment length polymorphisms corresponded with the results of serotyping by RIVM serumpools. Our results suggest that PCR-RFLP of the 5′ NCR of enteroviruses allows the rapid diagnosis and subtyping of enteroviruses, at least 7 kinds of above strains and is applicable for other echo and coxsackie viruses.

H303 Purification and Characterization of Alkaline Protease Produced by *Peudomonas* sp. BK7

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Pseudomonas sp. BK7 isolated from soil samples produced alkaline protease. The protease BK7 was purified by DEAE and Sephadex G-75 column chromatographies. The molecular weight of protease BK7 determind by gel filtration was 40,800. The optimum pH for the enzyme activity was 10 and the enzyme was stable from 5 to 11. The optimum temperature for the enzyme activity was 45℃ and about 50% of the original activity remained after incubation at 55℃ for 30min. The enzyme activity was completely inhibited by serine protease inhibitor, phenylmethanesulfonyl fluoride. Its proteolytic activity was not inhibited by EDTA and EGTA. The enzyme was stable toward sodium perborate, sodium triphosphate and some detergents such as sodium dodecyl sulfate and sodium linear alkylbenzene sulfonate..