DESIGN A NEW PRIMER FOR MOLECULAR IDENTIFICATION OF S. BOVIS BIOTYPES

Abstract:

The Streptococcus bovis group includes several species and subspecies of microorganisms that are associated with infection and colorectal cancer in human and animals. The objective of this study was to design new primer of S. bovis biotype. Specific primer was designed based on the conserved regions of the target genes used in the PCR assays. The size of PCR product was designed to obtain a staircase like pattern when separated by agarose gel electrophoresis. The conserved regions of these alignments were visualized using GeneDoc software, a specific primer pair from conserved region was designed to amplify the 16S rRNA. The designed primer was analyzed using BLAST, GenBank for specificity. The results showed that the designed PCR assay was 100% specific for their target gene with the correct PCR product size (398 bp). Streptococcus equinus strain 3141 16S ribosomal RNA gene, partial sequence 721 bp linear DNA. Streptococcus bovis S. cl. S.721(product=165 ribosomal RNA) LOCUS EU706410 721 bp. In conclusion, the specific primer was designed based on the conserved regions of the target genes used in the PCR assays product size (398 bp).

Material and Methods:

DNA sequence analysis: Specific primer was designed based on the conserved regions of the target genes used in the PCR assays. The size of PCR product was designed to obtain a staircase like pattern when separated by agarose gel electrophoresis. The conserved regions of these alignments were visualized using GeneDoc software, a specific primer pair from conserved region was designed to amplify the 16S rRNA. The specific primer was designed based on primer design guidelines where the primer length was between 18 to 30 bp, the annealing temperature as 2°C lower than the estimated melting temperature, the annealing temperature of each designed primer within the 55 to 75°C range, primer sequences selected was between 35% and 65% of G-C. The designed primer was analyzed using BLAST, GenBank for specificity.

One of the bacterial agents that has been found to be regularly associated with colorectal cancer is Streptococcus bovis [S. bovis]. S. bovis has been shown to have important impact on health since 25 to 80% of patients with S. bovis bacteremia have colorectal tumors and the incidence of association of colon neoplasia. It was shown that 94% of S. bovis bacteremia associated with colorectal cancer was in fact S. bovistbiotype I while only 18% was associated with biotype II. However, the objective of this study was to design new primer of S. bovis biotype isolated from colorectal cancer.

Results and Discussion:

The results showed that the designed PCR assay was 100% specific for their target gene with the correct PCR product size (398 bp). Streptococcus equinus strain 3141 16S ribosomal RNA gene, partial sequence 721 bp linear DNA. Streptococcus bovis S. cl. S.721(product=165 ribosomal RNA) LOCUS EU706410 721 bp. (Figure 1) However, subset is uniquely linked to a clinical presentation of gastrointestinal tract malignancy that showed PCR-positive stain. The biotype I-specific genes identified are predicted to be involved in the regulation of known streptococcal adhesion mechanisms. Whether this association is directly related to disease pathogenesis, the present study is directly link S. bovis with gastrointestinal tract, the biotype PCRs might prove to be useful markers in further investigations of the potential link between S. bovis and the development of GIT cancer. In conclusion, the specific primer was designed based on the conserved regions of the target genes used in the PCR assays product size (398 bp).

Identification of S. bovis by PCR Assay

Detection of S. bovis was carried out based on the 16S rRNA gene. PCR amplifications of approximately 398 bp in size as predicted by the Vector NTI software was observed from all the 3 reference strains. The specificity of the 16S rDNA PCR was tested with 5 other Gram-positive bacteria, 3 reference strains and all 77 clinical S. bovis isolates obtained from area of the study. The agarose gel electrophoresis result in figure 4.8, 4.9 and 4.10 clearly showed that the 16S rDNA primer successfully amplified only the gene from the S. bovis while other Gram-positive bacteria did not give any PCR amplicon (figure 2)

References:


