Use of temperature gradient gel electrophoresis to assess cultured Proteobacteria in clinical samples from Crohn's disease patients

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BACKGROUND

One of the hallmarks of gut dysbiosis in Crohn's disease (CD) is the elevation in the number of *Escherichia coli.* It is not clear whether there is some numerical variation in other Proteobacteria as well. A number of methods, such as qPCR, CFU counting and flow cytometry can be used for bacteria quantitation. Yet, none of these gives a panoramic view on the relative numerical variation in a bacterial community. In this work, we evaluated temperature gradient gel electrophoresis (TGGE) of amplicons of 16SrRNAV6 region as an alternative to these techniques to determine bacterial diversity in cultures from clinical samples of Crohn's disease patients.

METHODS

Gradient temperature.TGGE consisted in the separation of sequences of 16SV6rDNA amplicons under apreviously defined temperature gradient. This gradient was determined by testing a mixture of 16SV6rDNA amplicons, of 10 Proteobacteria(*E. coli, Klebsiella, Salmonella, Serratia, Shigella, Hafnia, Providencia, Morganella, Citrobacter, Campylobacter*) in a perpendicular run under a broad temperature variation (28ºC-49ºC) in a TGGE mini system (Biometra). The perpendicular run allowed the identification of the 42º-44ºC as the gradient for the best separation of the bands.

TGGE of pooled cultures.After determining the temperature gradient, four16SV6rDNA amplicons from MacConkey broth cultures of 4 distinct gut mucosal biopsies from a CD patient and an equivalent number from a control subject were submitted to TGGE in a parallel run, having the above Proteobacteria as controls.

Results and Discussion

TGGE separated the bands from eight of ten Proteobacteria tested, with good resolution. The exceptions were *Salmonella* and *E coli*, whose bands positioned much closed and could not always bedistinguished easily. The TGGE of 16SV6rDNA amplicons from each subject were very distinct. Regardless of the culture origin, TGGE profile of samples from the CD patient were usually restricted to bands corresponding to *Salmonella* and *Escherichia coli* whereas the profiles from control subjects showed at least 10 bands, some of which matching those used as reference controls . In addition, the bands from CD patients matching *Salmonella/E. coli* were denser than the equivalents in controls. PCR using primers for 3 *E. coli* O serogroups (O25, O83 and O126) using the patients cultures revealed the presence of O83 and O25, respectively in CD and control patients. These results indicate that TGGE can be used to assess the biodiversity of cultured Proteobacteria, since it displayed a clearly distinct band profile denoting a significant dysbiosis in the CD patient. In addition, *E. coli* O group determination revealed the presence of O83 only in cultures of CD patients. O83 is one of the typical serogroups of adherent and invasive *Escherichia coli*, the pathotype whose involvement with CD has been most suspected.

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