Ann Zootech (1996) 45, Suppl, 339

Molecular ecology of the human colonic microflora based on the utilization of ribosomal RNA-targeted hybridization probes

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The current knowledge of the human colonic microflora (over 400 species) is based on the phenotypic and genotypic characterization of culturable strains. It requires expertise in anaerobic culture techniques. The latter are delicate and lengthy and therefore have not allowed detailed studies of perturbations of microflora. In the meantime, comparative sequencing of ribosomal RNA has been applied to the phylogenetic positioning of organisms. Offering the advantages of ideal semantides (ubiquitous, mosaic in primary structure, with no reports of lateral transfer of genetic material...) characterized rRNAs constitute the most extensive database of homologous sequences (over 3000), which, within two years, should contain 16S rRNA sequences of all type strains in collection to date (DSM-ATCC concerted goal).

The identification of signature nucleotides in the rRNA sequences allows one to now design hybridization probes ranging in specificity from universal or the domain levels (Archaea, Bacteria, Eukaria) to the species or subspecies levels. We have used the rRNA sequence database to design a probe specific to the domain archaea, represented by methanogens in the human colon. This group of microorganisms ranges in population from absence to a significant presence in the dominant fecal flora (above 10^8/g for 30-60% of human population). After the work of Paster et al (1994, J Bacteriol, 176, 725-732), we have also designed a probe specific for the *Bacteroides-porphyromonas-Prevotella*-group comprising all species of the *B. fragilis* ensemble. These represent 30% of the normal human fecal flora. We have experimentally validated these and other probes such as a *Bifidobacterium*-specific probes proposed by Frothingham et al (1993, Microb Ecol Health Dis, 6, 23-27). This was done by hybridization with rDNA or total RNA obtained from pure cultures of microorganisms of the target groups and other members of the dominant normal human fecal flora (30 strains).

Two applications of these probes have been developed. Colony hybridization was used to enumerate total *Bacteroides* after growth on a non-selective medium under conditions enriching *Bacteroides* by elimination of extremely oxygen sensitive bacteria. To our knowledge, this is the first time a direct molecular counting of *Bacteroides* spp. is ever done. It nevertheless relies on culturability. It has been used in conjunction with *B. vulgatus*-specific monoclonal antibodies (G. Corthier), and has allowed us to get an insight into the structure of the *Bacteroides* population in humans from different age groups.

Quantitative hybridization using total RNA extracted from frozen fecal samples has also been used. Extensive work was undertaken to optimize extraction conditions. A frozen sample of 200 mg extracted by direct hot phenol-chloroform extraction in a 2.0 ml final volume (Stahl et al, 1988, Appl Environ Microbiol, 54, 1079-1084) gave the best results based on RNA quantity, quality and optimal recovery of the archaeal signal corresponding to *Methanobrevibacter* spp., i.e. microorganisms among the most difficult to lyse. The comparison of classical microbiological techniques and quantification by hybridization gave satisfactory results using archaea-specific domain-probes for the enumeration of methanogenic archaea. The wide range of population density in fecal samples allowed us to establish the threshold of detection as about 10^6/g. Above this limit, the two enumerations correlated well. In one case, the hybridization indicated a deficiency in culture technique for enumeration of all methanogens, suggesting the presence of non-culturable or distinct methanogens. Quantitative hybridization was also used in a test-study to measure the changing populations of total *Bacteroides* and *Bifidobacterium* spp. over a period of 50 days in the feces of a child aged 75 to 125 days. The results were expressed as relative signal (c.a. specific rRNA/total rRNA). Besides confirming population shifts extensively detailed in the literature, especially in relation to breast feeding, this work confirmed the efficiency of this method for investigating perturbations of components of a complex microbial flora over time or in response to chemical or microbial food additives. The use of frozen samples allows large studies to be carried out.