CHAPTER 11

The Molecular Biology of Bifidobacteria

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1 Summary

Bacteria belonging to the genus *Bifidobacterium* are amongst the most abundant species of the human and animal intestinal microflora and consequently, can play a fundamental role in the ecology of the gastrointestinal tract in both health and disease. However, attempts to improve the characteristics of bifidobacteria for practical applications, and gain a detailed knowledge of the physiology of these organisms, have been severely limited by the lack of molecular tools. Some progress made over the past decade, concerning characterisation of the bifidobacterial genes, the development of cloning vectors, and genetic transformation systems is discussed here. These developments now provide the basis for genetic approaches, that will eventually lead to a better understanding of these important organisms and the potential development of strains with improved characteristics.

2 Introduction

Bifidobacteria were first discovered in 1899 by Tissier at the Pasteur Institute, Paris, France. They are classified as Gram-positive, anaerobic, catalase negative, fermentative rods, often with a Y- or V- shaped morphology. The GC content of bifidobacteria is unusually high (usually over 55%) and consequently, they belong to a subclass of Gram-positive bacteria containing very GC-rich genomes (Scardovi, 1986). Other members of this high GC group include *Mycobacterium*, *Corynebacterium* and *Streptomyces*. From 16S rRNA analysis it is known that bifidobacteria are closely related to the actinomycete group and accordingly, the genus *Bifidobacterium* is included in the family *Actinomycetaceae* (Stanier *et al.*, 1987), rather than as part of the lactic acid group (e.g. *Lactobacillus* and *Lactococcus*)

Bifidobacteria are numerically important among the human and animal microbiota. In the human large intestine, for example, bifidobacteria can be present at concentrations of $10^{10}$ per gram of gut content (Mitsuoka, 1992) and may represent up to 25% of the culturable population of the gut microflora of the adult, and 95% of that in new-borns (Kawase et al., 1981) where their numbers are especially high in breast-fed infants. Among the major genera of human colonic bacteria, bifidobacteria are not considered to be pathogenic. Moreover, studies have concentrated upon the advantageous physiological functions of these bacteria in the human colon and it is now widely believed that bifidobacteria can have beneficial properties for their host. For example, established populations of *Bifidobacterium* are thought to form a barrier prohibiting the invasion of pathogens, either by the production of acids as metabolic end products, or via the excretion of other antibacterial compounds (Wang and Gibson, 1993). Other so-called probiotic effects include reducing the risk of cancer (Reddy and Rivenson, 1993) and modulating the immune response (Yasui et al., 1989).

Whilst some of the health promoting aspects of bifidobacteria remain speculative, their economic importance is unquestionable and there is increasing interest in the incorporation of these species into fermented milk products as probiotics. However, attempts to improve the characteristics of bifidobacteria for practical applications, and to gain a detailed knowledge of the physiology of these organisms have been severely limited by a lack of efficient molecular tools. Only in the last few years are molecular techniques, which have been applied successfully to the study of many other Gram-positive bacteria, finding application with bifidobacteria. The progress made over the past decade, concerning genetic developments in the genus *Bifidobacterium* is discussed in this chapter.

3 Genome size and structure

Pulse-field gel electrophoresis (PFGE) provides a means of separating DNA fragments of up to 700 kb in size and, thereby, provides a means of studying the organisation of bacterial genomes. The GC content of bifidobacteria is between 55 and 64 mol % (Scardovi, 1986) and, accordingly, restriction enzymes with recognition sequences containing only A and T nucleotides would be expected to cleave the genome at relatively few locations. Using PFGE, following the digestion of bifidobacterial DNA with such restriction endonucleases Bourget et al., (1993) have been able to produce physical maps of the genome of *Bifidobacterium breve* and have estimated the size of the genome to be approximately 2.1 Mb. This is 46% the size of the *Escherichia coli* genome, but is comparable to those of other bacteria producing lactic acid and their close relatives, the size of which varies between 1.7-2.4 Mb (Le Bougeois et al., 1991).

When the genomes of various strains of *B. breve* have been compared by PFGE significant heterogeneity is observed, when rare cleaving restriction enzymes are used to produce the electrophoretic profiles (Bourget et al., 1993). This raises the possibility that differences in restriction site distribution in the strains is due to significant chromosomal rearrangements such as translocations or inversions. PFGE has also been