Introduction

Bacteria have been present on earth for some 3.4 billion years, but the study of the evolution of bacteria is only decades old. Not that microbiologists lacked fascination for the evolution and phylogeny of bacteria. They shared the widely present human urge to classify living creatures. Early classification of plants and animals was based on comparative anatomy. Later on classification became phylogenetic, i.e. followed the lineages of the evolution. Bacteriologists have tried to apply the principles used in zoology to develop a phylogenetic system for bacteria. This was a failure (Van Niel, 1955) since the morphologic variation of bacteria is very limited: a sphere, a rod or a spiral. The only other criterion is the cell wall: either gram-negative or gram-positive.

A solution was indicated in 1965 by Zuckerkandl and Pauling in their paper 'Molecules as documents of evolutionary history' in which they argued that nucleic acid would be the master molecule, providing the basis for a molecular phylogeny. At that time it was still impossible to determine DNA and RNA sequences. When RNA sequencing became operational, Woese argued that the 16S ribosomal RNA (rRNA) subunit is well suited to measure phylogenetic relationships among bacteria. The pioneering work of Woese and coworkers demonstrated that 16S rRNA sequences are a proper basis for the phylogeny and evolution in bacteria (Woese *et al.*, 1983, Woese *et al.*, 1990). Indeed 16S rRNA sequences of bacteria have now become the basis of a phylogenetic classification system. Results presented in two chapters of this volume demonstrate that fluorescent probes to 16S rRNA can be used to enumerate the various phylogenetic groups of intestinal bacteria *in situ*.

Bacterial evolution and genetic exchange

To discriminate between closely related species, and certainly to follow subtle changes in the genome within a species, the sequence of other genes than those encoding rRNA have to be analyzed and compared. The first section of this volume focusses on the molecular mechanisms promoting and limiting the generation of genetic variation and gives a number of important, well-researched, examples of the effects of genomic changes in pathogenic bacteria.

The emphasis on pathogenic bacteria in this book, and the meeting on which it is based, is deliberate in view of the many selective pressures to which these

bacteria are exposed. These pressures shorten the time frame of evolution; often widespread changes are detected in bacteria isolated from the population within a few years after selection. In the analysis of the evolution of pathogenic bacteria one has to realize that, in the long era before these bacteria became pathogenic, they followed the 'normal' course of evolution. After that period, starting at the beginning of plant and animal life, a superimposed, more recent evolution took place. Selective factors that directed this evolution have been adaptation to symbiosis with the host and the immune selection of the host. Only very recently in this time scale more profound selective pressures, originating from human activities/civilization, came in force. Relevant factors are crowding of man and his domestic animals, changes in the environment, the use of antibiotics, vaccination. Some bacteria probably only became pathogenic after close contact with man (e.g. Vibrio).

Population genetics and clonal spread

Crowding would favor bacteria with virulence factors contributing to spread by diarrhea and coughing. Vaccination or natural immunity select for antigenic variants. Antibiotic resistance has been the sad consequence of improper use of these drugs. This volume gives several examples of genetic changes fitting in the above scenario. Gene transfer between bacteria appears to play an important role in acquiring and changing virulence factors and in the development of penicillin-resistant bacteria.

Throughout the years this ongoing evolution resulted in some bacterial clones that became so well-adapted to their hosts that they had an advantage over other clones of the same species. When such an adaptation coincides with pathogenicity it results in a rapid epidemic expansion of the clone followed by only a few additional mutations. Later on the population becomes heterogeneous (panmictic) again by horizontal gene exchange between bacteria. Some pathogens are not clonal. The third section of this book illuminates and explains the situation in this field.

Host-parasite interactions

Another major insight that is discussed is the interplay between pathogenic bacteria and their host cells. The middle part of this volume is devoted to this topic. One example is the very stable interaction between some insects and bacteria. Other reports focus on the dynamic interaction between bacteria and host cells. It is now clear that bacteria are continually monitoring their environment and answer the signals they receive by increasing or decreasing the transcription of genes. The activity of some genes is needed for survival and multiplication in the host. Five chapters demonstrate the powerful methods available to study host-

parasite interactions. One approach for the systematic identification of host-regulated bacterial genes is promoter-cloning followed by selection of differentially regulated promoters. Another is to use transposon mutagenesis. A very powerful modification of the latter method was reported at the meeting (Hensel *et al.*, 1995).

Future developments

Looking back over the unexpected scientific developments in the field of bacterial evolution during the last 10 years makes one cautious to predict too far ahead. But certain developments are already visible. First, new and reemerging infectious diseases will require attention. New outbreaks can lead to a better understanding of the evolution of virulence and the effects of human activity on this evolution. A new journal 'Emerging Infectious Diseases' focusses on these topics.

Second, it is now clear that the complete genomic DNA sequence of the most important pathogenic bacteria and a number of apathogenic bacteria will be available within 10 years. This will have an enormous impact on both the understanding of host-parasite interactions and the evolution of bacteria. Regions present in pathogenic bacteria which are absent in their apathogenic counterparts will be identified. Also the role and spread of 'pathogenicity islands', discrete segments of DNA that encode virulence traits (Hacker *et al.*, 1990), can be evaluated.

Bacterial evolution went on for more than 10^9 years before man arrived on the scene, and, about 10^4 years ago, started to affect the course of this evolution. The challenge will be to use our understanding of bacterial evolution and ecology in controlling infectious diseases.

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