Summary and concluding remarks

Microorganisms can readily adapt through various evolutionary processes and hence, they reside in a vast array of natural environments and sustain various biogeochemical cycles. Thus, understanding these cycles has hinged on our ability to characterize bacterial diversity. Several molecular techniques have been used for the assessment of bacterial diversity at different levels of resolution (i.e., genus, species or strain). Amongst these, comparisons of small subunit ribosomal RNA (16S rRNA) sequences and short oligonucleotides that hybridize to specific regions of rRNA molecules (e.g., fluorescent in situ hybridization, FISH) have become among the most powerful tools to assess the phylogenetic and functional diversity of microorganisms (Amann et al., 1995; Weisburg et al., 1991; Woese, 1987). The actual abundance and spatial distribution of many bacterial species in natural environments have been extensively studied by use of the FISH technique (for review, see Amann et al., 1995). However, to date, little is known about the abundance and spatial distribution of purple nonsulfur bacteria in natural environments despite the fact that this group of bacteria is widely distributed, and plays an important role in anaerobic nutrient cycles in many environments (Chapter 1). The natural habitats of purple nonsulfur bacteria are subject to substantial and frequent changes in environmental conditions (e.g., oxygen levels and light penetration) and these will influence the growth rate and physiological state of these bacteria. The sensitivity of FISH using 16S rRNA oligonucleotide probes is dependent on the cellular abundance of rRNA, and therefore on the cellular growth rates of purple nonsulfur bacterial populations in natural environments. The findings presented in Chapter 2 demonstrated the influence of different modes of growth and starvation on FISH in pure cultures of the purple nonsulfur bacterium *Rhodopseudomonas palustris*. This study shows that the detectability of individual cells using the FISH technique was strongly influenced by their physiological history and current physiological state. Interestingly, there were significant differences in metabolic activity and cell viability of *Rps. palustris* that had been grown and starved under oxic conditions in the dark, or under anoxic conditions in the light. The cell viability of *Rps. palustris* cells grown and starved under anoxic conditions in the light remained relatively high for a longer period of time as compared to cells that had been grown and starved under oxic conditions in the dark, or cells grown under anoxic conditions in the light and starved under anoxic conditions in the dark. This implies that light serves as a source of energy for maintaining cell viability in the absence of an external growth substrate.

Differences in the physiological characteristics of individual microorganisms typically result from genetic differences between the individual microorganisms. Such differences arise through evolutionary processes that select for variants with improved fitness under specific conditions. Given that many environments are carbon-limited, mutations that confer the ability to metabolize a xenobiotic compound can confer a strong selective advantage. This may account for the occurrence of microbial species able to metabolize anthropogenic compounds that have only recently been introduced to the environment. Many microbial species able to degrade xenobiotic compounds have been isolated from highly polluted environments (Herrick et al., 1997; Leahy and Colwell, 1990; Morris et al., 1992; van der Meer et al., 1998; Wu and Wiegel, 1997). While aerobic degradation of xenobiotic compounds has been extensively studied, little is known about the anaerobic mineralization of xenobiotic compounds. This might be due to the fact that such anaerobic bacteria are not easily cultivated, because they are rare in natural environments, or because these activities are only expressed under specific conditions.
Several studies in the past suggested that purple nonsulfur bacteria are likely to play an important role in mineralization of halogenated compounds in natural environments (Egland et al., 2001; Kamal and Wyndham, 1990; McGrath and Harfoot, 1997; Montgomery and Vogel, 1992; van der Woude et al., 1994). To better understand the potential of purple nonsulfur bacteria to degrade such compounds, the adaptive responses of *Rps. palustris* and the selection of novel metabolic ability in this species were investigated (Chapter 3). All of the *Rps. palustris* strains tested were originally able to metabolize 3-chlorobenzoate if benzoate was provided as a co-substrate. However, after long-term exposure to 3-chlorobenzoate the strains acquired the ability to metabolize this compound as the sole carbon source. The findings presented in Chapter 3 clearly demonstrate that adaptive mutations that expand the ability of *Rps. palustris* to metabolize xenobiotic compounds commonly and reproducibly occur although the mechanisms responsible for the adaptive changes remain unknown. The frequent occurrence of such adaptive mutations, may well account for the metabolic versatility of *Rps. palustris* that is widely recognized (Gibson and Harwood, 1995; Harwood and Gibson, 1988; Sasikala and Ramana, 1998).

Genetic variation is a prerequisite of biological evolution. The amount and kind of variation found in populations apparently differs depending on the life history of microorganisms, on the frequency of various mutagenic processes, and differences in selective pressures imposed by various habitats. Hence, many species of bacteria in the environment, particularly in soil, have been shown to compose of genetically distinct and diverse clones that are well adapted to local conditions (Cho and Tiedje, 2000; Fulthorpe et al., 1998; Istock et al., 1992; Johnsen et al., 1996; Maynard Smith et al., 1993; McArthur et al., 1988; Wise et al., 1995). In Chapter 4, the phenotypic and genetic characteristics of *Rps. palustris* strains originating from various ecological sources were reported. While all strains studied had certain phenotypic characters in common, including the ability to metabolize benzoate and degrade 2- and 3-chlorobenzoate, there were also significant differences among the strains (Table I in Chapter 4). Phylogenetic and genetic characterization based on gene sequences of 16S rRNA and form II ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) genes, as well as rep-PCR genomic DNA fingerprinting revealed differences among the strains. Interestingly, all analyses showed the existence of three distinct clades. These clades may have developed through adaptive evolution of *Rps. palustris* clones to prevailing specific environmental conditions and this may represent an incipient sympatric speciation event.

While the results presented in Chapter 4 demonstrated the existence of significant phenotypic and genotypic diversity among strains of *Rps. palustris*, the apparent extent of diversity within this species may be distorted by the fact that the strains used in this study were isolated from selective enrichment cultures (Dunbar et al., 1997). To expand our knowledge of the extent of diversity within species of purple nonsulfur bacteria, Chapter 5 described the phylogenetic, genotypic, and phenotypic characteristics of 128 strains isolated by direct plating of sediment samples taken from 2 ecologically distinct sites, Haren and De Biesbosch, The Netherlands. Strikingly, BOX-PCR genomic DNA fingerprinting revealed there were 60 distinct genotypes. Analyses of 16S rRNA gene sequences of representatives from each genotype showed that there were 5 and 8 different species of purple nonsulfur bacteria from the Haren and the Biesbosch sites, respectively. At the Haren site, 81% of the isolates were identified as *Rps. palustris*, whereas clones of *Rhodoferax fermentans* were numerically dominant at the Biesbosch site and comprised 46% of the total isolates. The genomic fingerprints of *Rps. palustris* strains from the 2 sampling sites were significantly different. None of the genotypes found in the Haren site was found in the Biesbosch site.
These results imply that certain strains may be endemic to each sampling site. The results of the study also show that the genotypic diversity within the species *Rps. palustris* at the Biesbosch site was significantly higher than that of the Haren site. If as postulated by McArthur et al. (1988), the genetic diversity of species increases with habitat variability, our findings of significant differences in genotypic diversity between the 2 sampling sites suggest that the Biesbosch site exhibits a greater habitat variability than the Haren site. Surprisingly, not all strains of *Rps. palustris* isolated from these sites could degrade benzoate in spite of the fact that this trait is reportedly characteristic of the species (Gibson and Harwood, 1995; Imhoff and Trüper, 1992).

Studies of bacterial biogeography are needed to better understand current population structures and their evolutionary history. However, to date, little is known about the biogeography of free-living bacteria in natural environments. This is in contrast to the many plant-, animal-, and human-associated bacterial species (see Chapter 1). In Chapter 6, the biogeography of *Rps. palustris* strains isolated from aquatic sediments was investigated. Sediment samples were collected along a linear 10 m transect, and 30 clones from each of 5 sampling locations were characterized by BOX-PCR genomic DNA fingerprinting. Cluster analysis of 150 genomic fingerprints showed there were 4 major genotypes of *Rps. palustris* (A, B, C, and D, Fig. 2 in Chapter 6) and each genotype existed in defined patterns of distribution. The differences in genomic fingerprint patterns among the 4 major genotypes of *Rps. palustris* were accompanied by the differences in phenotypic characteristics. These results indicate that each genotype is a distinct clade and functional differences that have ecological significance may exist among these clonal lineages. Moreover, the clonal lineages observed along the sampling transect may represent ecotypes that have diverged through sympatric speciation. Morisita-Horn similarity coefficients (Stiling, 1999) that compare the numbers of common genotypes found in pairs of sampling locations showed that there was substantial similarity between locations that were 1 cm apart ($C_{MH} = 0.97$), but there was almost no similarity between locations that were $\geq 9$ m apart ($C_{MH} \leq 0.25$). These calculations showed a gradual decrease in similarity among the 5 locations as a function of distance, indicating that natural populations of *Rps. palustris* are assemblages of genetically distinct ecotypes and the distribution of each ecotype is patchy in its distribution.

While the outcome of Chapters 4, 5, and 6 yielded a significant insight into the genotypic and phenotypic diversity, and biogeography of natural populations of *Rps. palustris*, further research is needed to answer the ecological significance of the observed patterns of genotypic and phenotypic diversity among local populations of *Rps. palustris*.

References


