Effect of Transient Oxic Conditions on the Composition of the Nitrate-Reducing Community from the Rhizosphere of *Typha angustifolia*

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**Abstract.** Within a nitrate-reducing bacterial community, a niche differentiation between denitrifying and nitrate ammonifying bacteria may be determined by a complex of environmental parameters, such as the availability of carbon, nitrate, and oxygen. Hence, oxygen- and carbon-releasing aerenchymatous plants may affect the composition of the nitrate-reducing community in waterlogged sediment. The composition of the nitrate-reducing community in the rhizosphere of the aerenchymatous plant species *Typha angustifolia* was compared with the community in nonrhizospheric sediment. All three functional groups (NO$_2^-$ accumulators, N$_2$O producers, and presumed NH$_4^+$ producers) were present at both sites with a ratio of 36:45:12 and 43:22:18 for nonrhizospheric and rhizospheric sediments, respectively. Most of the isolated were gram-negative, and approximately 50% of these strains demonstrated an obligatory oxidative metabolism.

In the absence of nitrate, *Enterobacteriaceae* (belonging to the NO$_2^-$ accumulating group) became dominant during enrichment of bacteria from the rhizosphere of *T. angustifolia* in a chemostat with glycerol (20 mM) as substrate, both under strictly anoxic and transient oxic conditions. Addition of nitrate to the chemostats led to the predominance of denitrifying pseudomonads, irrespective of the presence or absence of oxygen. However, in the presence of nitrate under anoxic conditions, enterobacteria persisted in the medium together with pseudomonads.

It was concluded that oxidative bacteria such as pseudomonads are the better competitors for limiting amounts of glycerol, provided oxygen or nitrate is present. In the absence of these electron acceptors, fermentative bacteria become dominant.
Introduction

Reduction of nitrate in anaerobic environments occurs by two dissimilatory processes: respiratory denitrification and dissimilatory nitrate reduction to ammonium [19]. During both processes nitrate is reduced first to nitrite. Denitrifying bacteria reduce nitrate further to gaseous endproducts, i.e., NO, N₂O and N₂, whereas nitrate ammonifiers produce an almost equivalent amount of ammonium from the intermediate nitrite. The predominance of either one of these nitrate-reducing types will influence the availability of inorganic nitrogen in the environment. The bacterial communities participating in such processes consist of various genera [2]. Generally, it appears that in nitrate ammonification, facultative, or obligate anaerobic fermentative bacteria, such as Bacillus spp. [23], Escherichia coli [5], Klebsiella spp. [5], Citrobacter sp. [15], Vibrio spp. [13] or Clostridium butyricum [10], are involved. In contrast, denitrification usually is due to the activities of oxidative organisms such as Pseudomonas spp. [3, 8, 22], Paracoccus denitrificans [3] and Alcaligenes sp. [3, 22], which are capable of growing either aerobically or anaerobically by respiring oxygen or nitrate, respectively. It has been suggested that the major factor controlling the predominance of either the denitrifying or ammonium-producing populations is their ability to compete for organic substrates irrespective of habitat [21]. Tiedje et al. [22] postulated that the ratio of available carbon to electron acceptor determines whether nitrate is used for dissimilatory ammonification or for denitrification. At a high ratio, the electron acceptor capacity would be limited, which favors those organisms not entirely dependant on external electron acceptors, i.e., nitrate ammonifiers. However, when the ratio is low, those organisms that gain most energy per electron acceptor, i.e., the denitrifying bacteria, would be favored.

In addition, oxygen itself might be an important environmental parameter in selecting the dominant nitrate-reducing populations, as anaerobic growth of nitrate-reducing bacteria in the absence of nitrate is only possible in organisms with a potentially fermentive metabolism. Under permanent anoxic conditions the ammonium producers may be favored. Hence, the occurrence of denitrifying and ammonifying populations in situ might be determined by interacting environmental factors [4, 14]. So, aerenchymatous plants growing in submerged anaerobic sediments, such as Typha angustifolia, may affect the predominance of either denitrifying or nitrate-ammonifying bacteria in the rhizosphere in two ways, i.e., by the production of carbon and by leakage of oxygen.

In this paper, the composition of the nitrate-reducing bacterial community in the rhizosphere of T. angustifolia was compared with the community in the bulk of the sediment. In addition, enrichments of dominant populations have been made from natural mixed bacterial communities of the rhizosphere of T. angustifolia in continuous cultures with different C/N ratios both under anoxic and transient oxic conditions. A chemostat was used for these experiments, as it enables enrichments under well defined conditions of carbon, nitrate, and/or oxygen limitation. Glycerol was chosen as substrate for the chemostat enrichment experiments because diverse bacterial species are unable to ferment it in the absence of external electron acceptors [12, 18], and this compound