THE RESPIRATORY NITRATE REDUCTASE IS IMPORTANT TO A SUCCESSFUL *Herbaspirillum seropedicae*-WHEAT INTERACTION

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The nitrogen-fixing β-proteobacterium *Herbaspirillum seropedicae* is found in association with gramineous plants of economic importance, including wheat, and maize. Nitrate is the main inorganic nitrogen source found in soil, however its metabolism in *H. seropedicae* is not well understood. Bacteria can incorporate nitrate through nitrate reductases, which reduces nitrate to nitrite. *H. seropedicae* has putative genes encoding two nitrate reductases: the respiratory nitrate reductase (hereafter NAR) and the assimilatory nitrate reductase (hereafter NAS). The aim of this study was to investigate the roles of NAR and NAS enzymatic complexes of *H. seropedicae*. A mutant for the catalytic site of NAS (*nasA*$^-$ mutant) cannot grow on nitrate as the only nitrogen source, suggesting that NAS is the main pathway for nitrate assimilation in *H. seropedicae*. Moreover, a mutant for the catalytic site of NAR (*narG*$^-$ mutant) can grow well on nitrate as the only nitrogen source. In addition, *H. seropedicae* is not able to grow anaerobically in the presence of nitrate. These results suggest that NAR of this bacterium cannot use nitrate as the terminal electron acceptor nor it is required for nitrate assimilation. We have also determined the carbon consumption of *H. seropedicae* wild type and *narG*$^-$ strains growing in the presence of ammonium or nitrate. The wild type strain consumed higher amounts of carbon in the presence of nitrate as compared with ammonium; however, the carbon consumption by the *narG*$^-$ mutant was similar with ammonium or nitrate, suggesting that the high consumption of carbon by NAR is important to recycle NADH. In addition, the NO production was decreased in *narG*$^-$ when compared with the wild type strain. Together, these results suggest that NAR is involved with both higher use of carbon sources and NO production. Considering the probable NO involvement with bacteria-plant association, the *H. seropedicae* interaction with wheat was also investigated using the wild type and *narG*$^-$ strains. The dry weight of the stem of plants inoculated with *H. seropedicae* was increased as compared with non-inoculated plants (p= 0.11). In addition, plants inoculated with the *narG*$^-$ mutant showed a decrease in dry weight of stem compared to those inoculated with wild type strain (p= 0.00011) or non-inoculated plants (p= 0.015) suggesting the importance of NAR in the *H. seropedicae*-wheat interaction. Possibly this is due to NO production by NAR.

Keywords: *Herbaspirillum seropedicae*, nitrate, plant-bacterium interaction, wheat