A Study of Peroxide Resistance in the Microaerophile, *Spirillum volutans*

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A STUDY OF PEROXIDE RESISTANCE IN THE MICROAEROPHILE, *SPIRILLUM VOLUTANS*

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(ABSTRACT)

Studies of adaptive responses of the microaerophile *Spirillum volutans* to various stresses such as heat and peroxide shock have been hampered by an inability to obtain reliable colony counts of the organism by the spread plate method. Colony counts approaching direct microscopic counts (DMCs) were obtained by inoculating culture dilutions into a semisolid version of the medium and by supplementing the medium with pyruvate, which destroys hydrogen peroxide. Use of the new overlay/pyruvate method for colony counts revealed that exposure of *S. volutans* to 40 degrees C for 100 min results in a greater survival at 45 degrees C compared with cells having no prior exposure to 40 degrees C. *Spirillum volutans* is catalase-negative and is rapidly killed by levels of H_2O_2 greater than 10 micromolar. A mutant isolated by single step mutagenesis with diethyl sulfate was able to survive and grow after exposure to 40 micromolar H_2O_2 and was effective in eliminating H_2O_2 concentrations added to the medium. In addition, the mutant had high NADH peroxidase activity (0.072 I.U. mg^{-1}) whereas the wild type had no detectable activity (<0.0002 I.U. mg^{-1}). Nevertheless, the mutant was no more tolerant to O_2 than the wild type. NADH peroxidase activity has not previously been reported in bacteria having a strictly respiratory type of metabolism. The peroxide-resistant mutant constitutively expresses a 21.5 kDa protein as determined by one and two-dimensional PAGE. This protein was undetectable and noninducible in the wild type cells. Part of the gene that encodes the protein was cloned by using amino acid sequence data obtained by both mass spectrometry and NH_2-terminal sequencing. The deduced 158 amino acid polypeptide showed high similarity to rubrerythrin and nigerythrin previously described in

the anaerobes *Clostridium perfringens* and *Desulfovibrio vulgaris* and to putative rubrerythrin proteins found in some anaerobic archeons. This is the first report of this type of protein in an organism that must respire with oxygen. This rubreythrin-like protein may play a role in the peroxide resistance of the mutant. The methodology may be useful for rapid cloning of genes in other bacteria.