Characterization of novel nitrate reductase-deficient mutants for transgenic *Dunaliella salina* systems

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**ABSTRACT.** The aim of the present study was to isolate and characterize novel nitrate reductase (NR)-deficient mutants, which may be useful for the transgenic manipulation of *Dunaliella salina*. Three NR-deficient mutants of *D. salina*, J-1, J-2, and J-3, were successfully isolated by screening for chlorate resistance after chemical mutagenesis with ethylnitrosourea. NR activity was not detected in the mutants and the expression of NR mRNA was significantly decreased. Growth analysis of *D. salina* strains grown in media containing different nitrogen sources revealed that these mutants were capable of utilizing nitrite and urea, but not nitrate as a nitrogen source, indicating that these mutants are indeed NR-deficient. Mutation analysis of NR cDNA sequences revealed that there were 11 point mutations shared by the J-1, J-2, and J-3 mutants. Furthermore, the results of the functional complementation experiment showed that NR activity of transformant T-1 derived from J-1 was recovered to 48.1% of that of the wild-type *D. salina*. The findings of the present study indicate that nitrate may be used as a
selective agent rather than antibiotics or herbicides for the isolated NR-deficient mutants in future transgenic *D. salina* systems.

**Key words:** *Dunaliella salina*; Nitrate reductase; Mutant; Transgene