**442g Orthric Rieske Dioxygenases for Degrading Aromatic Pollutants**

*Thomas K. Wood, Brendan G. Keenan, and Thammajun Leungsakul*

Since mixtures of pollutants are frequently encountered, a hybrid dioxygenase system was created here that degrades simultaneously 0.1 mM 2,4-dinitrotoluene (24DNT) and 0.1 mM naphthalene by combining the terminal oxygenase genes (*dntAcAd*) of the 2,4-dinitrotoluene dioxygenase (R34DDO) from *Burkholderia cepacia* R34 with the electron transport and terminal oxygenase genes (*nagAaAbAcAd*) of the naphthalene dioxygenase (NDO) from *Ralstonia* sp. strain U2 (NDO-R34DDO). Neither NDO nor R34DDO alone had significant activity on 24DNT or naphthalene, respectively. NDO-R34DDO also degraded 0.1 mM 4-nitrotoluene 5-fold faster than R34DDO alone and 1.5-fold faster than NDO alone, indicating that the novel NDO-R34DDO dioxygenase could also oxidize additional substrates with high proficiency. The terminal oxygenase genes (*nbzAcAd*) of the nitrobenzene dioxygenase (NBDO) from *Comamonas* sp. strain JS765 were also combined with the *dntAaAbAcAd* genes of R34DDO, generating R34DDO-NBDO, and the *dntAcAd* genes of DNTDDO from *Burkholderia* sp. strain DNT were combined with the *nagAaAb* genes of NDO and *nbzAcAd* genes of NBDO, generating NDO-NBDO-DNTDDO, to create hybrid dioxygenases with the ability to simultaneously degrade the 2,4,6-trinitrotoluene (TNT) reduction products 2-amino-4,6-dinitrotoluene (2A46DNT) and 4A26DNT. During simultaneous incubation with 0.25 mM 2A46DNT and 0.25 mM 4A26DNT, R34DDO-NBDO generated 3-amino-4-methyl-5-nitrocatechol (3A4M5NC) and 2-amino-4,6-dinitrobenzyl alcohol (2A46DNBA) from 2A46DNT 4-fold and 3-fold faster, respectively, than NBDO alone and formed 3A6M5NC from 4A26DNT (R34DDO alone has no activity on 4A26DNT). Therefore, the addition of NBDO *nbzAcAd* to R34DDO allows for a more balanced degradation of the TNT reduction products. The novel construct NDO-NBDO-DNTDDO generated 3A6M5NC from 4A26DNT 3-fold faster than NBDO alone. This is the first report to describe a dioxygenase system capable of simultaneously degrading mixtures of 24DNT and naphthalene and mixtures of 2A46DNT and 4A26DNT and to investigate the compatibility of the ferredoxin subunit from a Rieske non-heme iron dioxygenase with a dual terminal dioxygenase system.