

# Phosphorothioated DNA Is Shielded from Oxidative Damage

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## ABSTRACT

Phosphorothioated DNA (PT-DNA) is a synthetic DNA derivative that has been widely used in molecular biology. However, the stability of PT-DNA under oxidative conditions remains unclear. In this study, we investigated the oxidative stability of PT-DNA in *Salmonella enterica*, *Escherichia coli*, and *Pseudomonas fluorescens*. The results showed that PT-DNA was significantly more stable than natural DNA under oxidative conditions. The half-life of PT-DNA in *S. enterica* was  $10.58 \pm 0.90$  min, while the half-life of natural DNA was  $2.419 \pm 0.59$  min. In *E. coli*, the half-life of PT-DNA was  $31.03 \pm 1.21$  min, and the half-life of natural DNA was  $1.21 \pm 0.15$  min. In *P. fluorescens*, the half-life of PT-DNA was  $10.1 \pm 0.5$  min, and the half-life of natural DNA was  $1.1 \pm 0.1$  min. These results indicate that PT-DNA is more stable than natural DNA under oxidative conditions, which may be due to the presence of sulfur in the phosphate backbone of PT-DNA.

## IMPORTANCE

Phosphorothioated DNA (PT-DNA) is a synthetic DNA derivative that has been widely used in molecular biology. However, the stability of PT-DNA under oxidative conditions remains unclear. In this study, we investigated the oxidative stability of PT-DNA in *Escherichia coli*. The results showed that PT-DNA was significantly more stable than natural DNA under oxidative conditions. The half-life of PT-DNA in *E. coli* was  $31.03 \pm 1.21$  min, while the half-life of natural DNA was  $1.21 \pm 0.15$  min. These results indicate that PT-DNA is more stable than natural DNA under oxidative conditions, which may be due to the presence of sulfur in the phosphate backbone of PT-DNA.

## KEYWORDS

phosphorothioated DNA, oxidative damage, stability, *Escherichia coli*

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(1). A  
 (2-4).  
 (5, 6). *Clostridium*  
*difficile*, 60% (7).  
*Leptospira* (8), A 50%  
*Mycobacterium abscessus* (9, 10).  
 (5, 6),  
*Escherichia coli* 7A, *Salmonella enterica*  
 87, 10<sup>4</sup> ( )  
 (5). 10<sup>3</sup>  
*Vibrio* (5). A  
 A (11).  
 5' 3' *E. coli* *Streptomyces lividans* AA /  
 (12).  
 A  
 A *in vitro* (13, 14).  
 (14-16),  
*E. coli* *S. enterica*  
 (16). A  
 (16).  
*E. coli* ( )  
 (17-19).  
 A ( ) A.  
 ( )  
 A

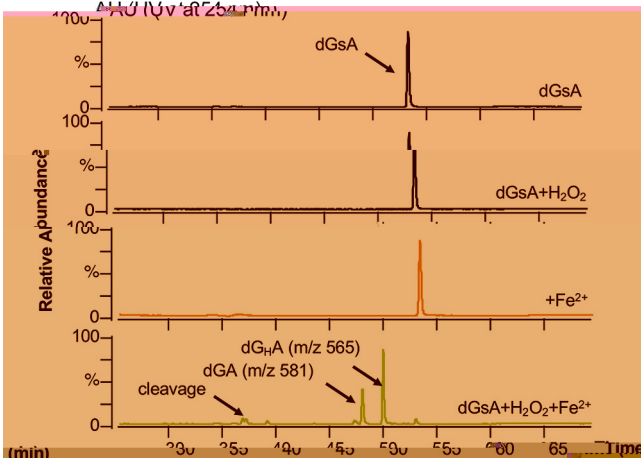
**RESULTS**

**Fenton reaction of PT DNA.**

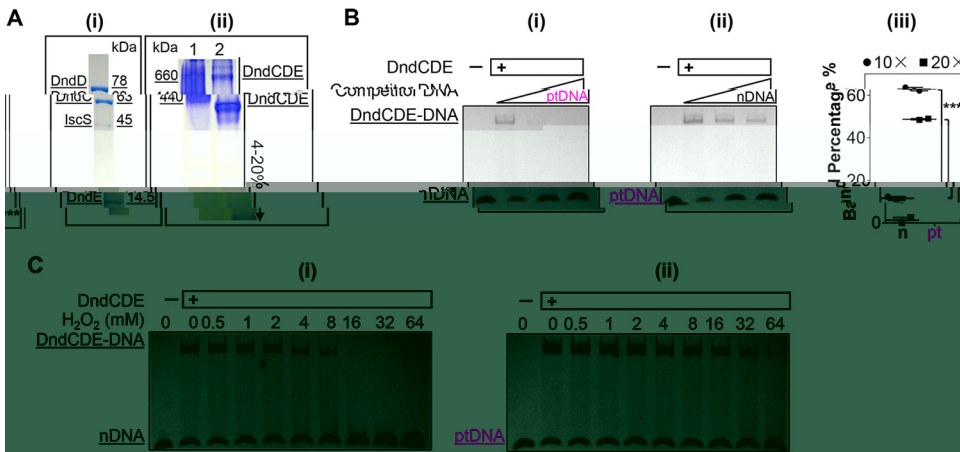
$\text{Fe}^{2+}$  (100  $\mu\text{M}$ )  
 ( )  
 A ( )  
 A (m/z 581) A (m/z 565) (15).  
 A (13),  
 A  
 100  $\mu\text{M}$

**Preferential complexation of DndCDE to PT DNA.**

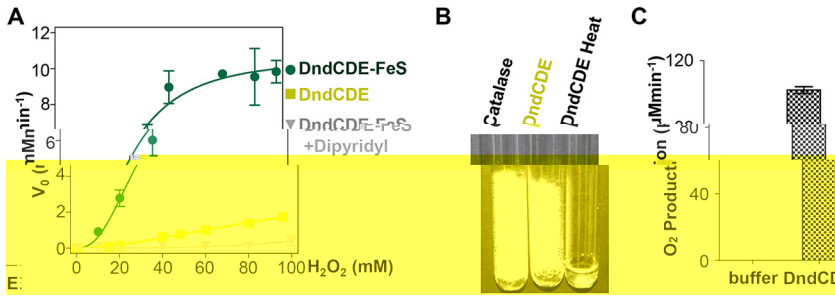
A  
*E. coli*  
 ( ) 1655 (17). A  
*in vivo*  
*S. enterica* 87 *E. coli*.  
*dndCDE*



**FIG 1** HPLC chromatograms showing the oxidation of dGsA. The top three panels show dGsA, dGsA+H<sub>2</sub>O<sub>2</sub>, and dGsA+H<sub>2</sub>O<sub>2</sub>+Fe<sup>2+</sup> respectively. The bottom panel shows the oxidation of dGsA+H<sub>2</sub>O<sub>2</sub>+Fe<sup>2+</sup> with peaks for dG<sub>4</sub>A (m/z 565), dGA (m/z 581), and cleavage products. The x-axis is Time (min) from 20 to 65, and the y-axis is Relative Abundance (%).



**FIG 2** Gel electrophoresis and quantification of DNA damage. Panel A shows SDS-PAGE of DndCDE and DndCDE-DNA. Panel B shows agarose gel electrophoresis of DNA damage with a bar graph of B-IP Percentage. Panel C shows agarose gel electrophoresis of DNA damage with increasing H<sub>2</sub>O<sub>2</sub> concentrations.

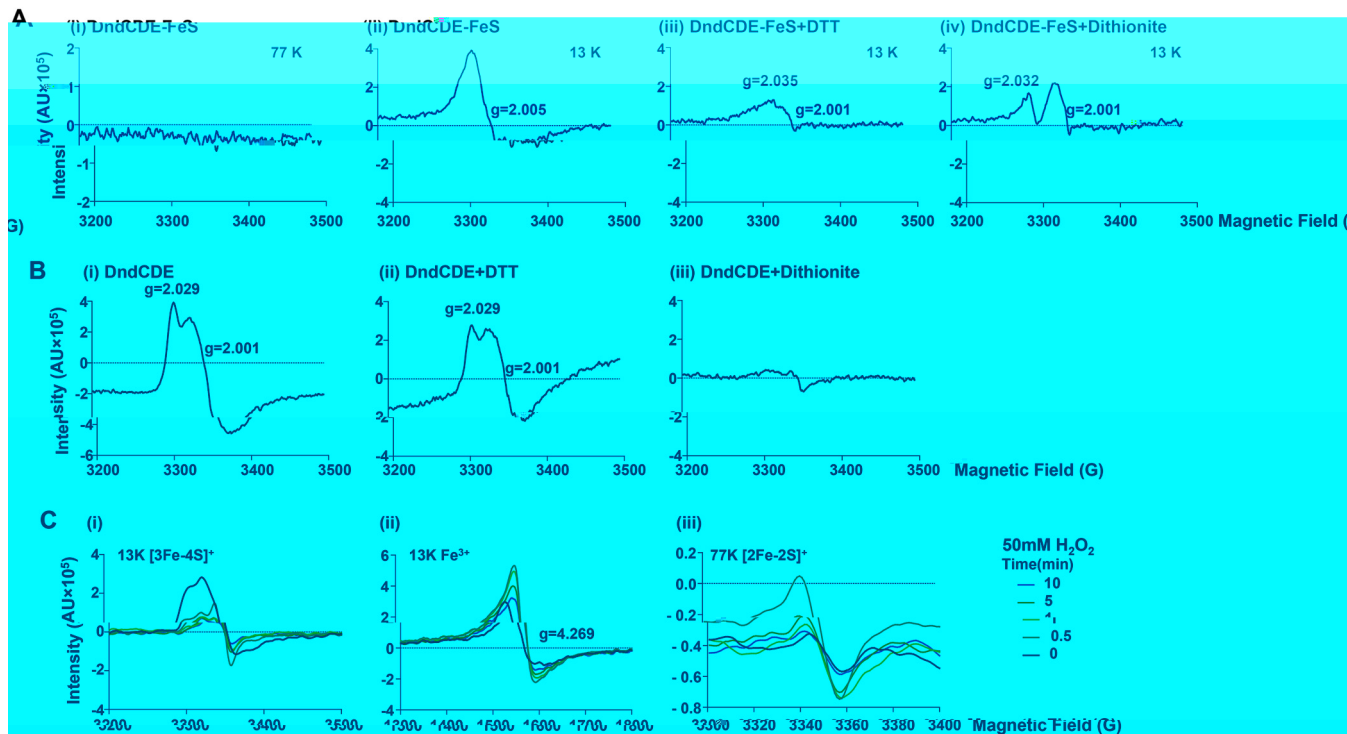


**FIG 3** (A)  $V_0$  vs  $H_2O_2$  concentration for DndCDE-FeS (green circles), DndCDE (yellow squares), and DndCDE-FeS + Dipyridyl (grey triangles). (B) Test tubes showing catalase activity in DndCDE and DndCDE Heat. (C) Bar chart showing  $O_2$  production for buffer and DndCDE. Error bars represent standard deviation ( $n=3$ ).

**DndCDE-FeS actively decomposes  $H_2O_2$ .**

(20, 21). (22). (23), (24, 25), (26). (27). (28). (29). (30). (31). (32). (33). (34). (35). (36). (37). (38). (39). (40). (41). (42). (43). (44). (45). (46). (47). (48). (49). (50). (51). (52). (53). (54). (55). (56). (57). (58). (59). (60). (61). (62). (63). (64). (65). (66). (67). (68). (69). (70). (71). (72). (73). (74). (75). (76). (77). (78). (79). (80). (81). (82). (83). (84). (85). (86). (87). (88). (89). (90). (91). (92). (93). (94). (95). (96). (97). (98). (99). (100). (101). (102). (103). (104). (105). (106). (107). (108). (109). (110). (111). (112). (113). (114). (115). (116). (117). (118). (119). (120). (121). (122). (123). (124). (125). (126). (127). (128). (129). (130). (131). (132). (133). (134). (135). (136). (137). (138). (139). (140). (141). (142). (143). (144). (145). (146). (147). (148). (149). (150). (151). (152). (153). (154). (155). (156). (157). (158). (159). (160). (161). (162). (163). (164). (165). (166). (167). (168). (169). (170). (171). (172). (173). (174). (175). (176). (177). (178). (179). (180). (181). (182). (183). (184). (185). (186). (187). (188). (189). (190). (191). (192). (193). (194). (195). (196). (197). (198). (199). (200). (201). (202). (203). (204). (205). (206). (207). (208). (209). (210). (211). (212). (213). (214). (215). (216). (217). (218). (219). (220). (221). (222). (223). (224). (225). (226). (227). (228). (229). (230). (231). (232). (233). (234). (235). (236). (237). (238). (239). (240). (241). (242). (243). (244). (245). (246). (247). (248). (249). (250). (251). (252). (253). (254). (255). (256). (257). (258). (259). (260). (261). (262). (263). (264). (265). (266). (267). (268). (269). (270). (271). (272). (273). (274). (275). (276). (277). (278). (279). (280). (281). (282). (283). (284). (285). (286). (287). (288). (289). (290). (291). (292). (293). (294). (295). (296). (297). (298). (299). (300). (301). (302). (303). (304). (305). (306). (307). (308). (309). (310). (311). (312). (313). (314). (315). (316). (317). (318). (319). (320). (321). (322). (323). (324). (325). (326). (327). (328). (329). (330). (331). (332). (333). (334). (335). (336). (337). (338). (339). (340). (341). (342). (343). (344). (345). (346). (347). (348). (349). (350). (351). (352). (353). (354). (355). (356). (357). (358). (359). (360). (361). (362). (363). (364). (365). (366). (367). (368). (369). (370). (371). (372). (373). (374). (375). (376). (377). (378). (379). (380). (381). (382). (383). (384). (385). (386). (387). (388). (389). (390). (391). (392). (393). (394). (395). (396). (397). (398). (399). (400). (401). (402). (403). (404). (405). (406). (407). (408). (409). (410). (411). (412). (413). (414). (415). (416). (417). (418). (419). (420). (421). (422). (423). (424). (425). (426). (427). (428). (429). (430). (431). (432). (433). (434). (435). (436). (437). (438). (439). (440). (441). (442). (443). (444). (445). (446). (447). (448). (449). (450). (451). (452). (453). (454). (455). (456). (457). (458). (459). (460). (461). (462). (463). (464). (465). (466). (467). (468). (469). (470). (471). (472). (473). (474). (475). (476). (477). (478). (479). (480). (481). (482). (483). (484). (485). (486). (487). (488). (489). (490). (491). (492). (493). (494). (495). (496). (497). (498). (499). (500). (501). (502). (503). (504). (505). (506). (507). (508). (509). (510). (511). (512). (513). (514). (515). (516). (517). (518). (519). (520). (521). (522). (523). (524). (525). (526). (527). (528). (529). (530). (531). (532). (533). (534). (535). (536). (537). (538). (539). (540). (541). (542). (543). (544). (545). (546). (547). (548). (549). (550). (551). (552). (553). (554). (555). (556). (557). (558). (559). (560). (561). (562). (563). (564). (565). (566). (567). (568). (569). (570). (571). (572). (573). (574). (575). (576). (577). (578). (579). (580). (581). (582). (583). (584). (585). (586). (587). (588). (589). (590). (591). (592). (593). (594). (595). (596). (597). (598). (599). (600). (601). (602). (603). (604). (605). (606). (607). (608). (609). (610). (611). (612). (613). (614). (615). (616). (617). (618). (619). (620). (621). (622). (623). (624). (625). (626). (627). (628). (629). (630). (631). (632). (633). (634). (635). (636). (637). (638). (639). (640). (641). (642). (643). (644). (645). (646). (647). (648). (649). (650). (651). (652). (653). (654). (655). (656). (657). (658). (659). (660). (661). (662). (663). (664). (665). (666). (667). (668). (669). (670). (671). (672). (673). (674). (675). (676). (677). (678). (679). (680). (681). (682). (683). (684). (685). (686). (687). (688). (689). (690). (691). (692). (693). (694). (695). (696). (697). (698). (699). (700). (701). (702). (703). (704). (705). (706). (707). (708). (709). (710). (711). (712). (713). (714). (715). (716). (717). (718). (719). (720). (721). (722). (723). (724). (725). (726). (727). (728). (729). (730). (731). (732). (733). (734). (735). (736). (737). (738). (739). (740). (741). (742). (743). (744). (745). (746). (747). (748). (749). (750). (751). (752). (753). (754). (755). (756). (757). (758). (759). (760). (761). (762). (763). (764). (765). (766). (767). (768). (769). (770). (771). (772). (773). (774). (775). (776). (777). (778). (779). (780). (781). (782). (783). (784). (785). (786). (787). (788). (789). (790). (791). (792). (793). (794). (795). (796). (797). (798). (799). (800). (801). (802). (803). (804). (805). (806). (807). (808). (809). (810). (811). (812). (813). (814). (815). (816). (817). (818). (819). (820). (821). (822). (823). (824). (825). (826). (827). (828). (829). (830). (831). (832). (833). (834). (835). (836). (837). (838). (839). (840). (841). (842). (843). (844). (845). (846). (847). (848). (849). (850). (851). (852). (853). (854). (855). (856). (857). (858). (859). (860). (861). (862). (863). (864). (865). (866). (867). (868). (869). (870). (871). (872). (873). (874). (875). (876). (877). (878). (879). (880). (881). (882). (883). (884). (885). (886). (887). (888). (889). (890). (891). (892). (893). (894). (895). (896). (897). (898). (899). (900). (901). (902). (903). (904). (905). (906). (907). (908). (909). (910). (911). (912). (913). (914). (915). (916). (917). (918). (919). (920). (921). (922). (923). (924). (925). (926). (927). (928). (929). (930). (931). (932). (933). (934). (935). (936). (937). (938). (939). (940). (941). (942). (943). (944). (945). (946). (947). (948). (949). (950). (951). (952). (953). (954). (955). (956). (957). (958). (959). (960). (961). (962). (963). (964). (965). (966). (967). (968). (969). (970). (971). (972). (973). (974). (975). (976). (977). (978). (979). (980). (981). (982). (983). (984). (985). (986). (987). (988). (989). (990). (991). (992). (993). (994). (995). (996). (997). (998). (999). (1000).

**$H_2O_2$  decomposition requires an intact DndCDE.**



**FIG 4** EPR spectra of DndCDE-FeS and related complexes. (A) EPR spectra of DndCDE-FeS at 77 K and 13 K, and DndCDE-FeS+DTT and DndCDE-FeS+Dithionite at 13 K. (B) EPR spectra of DndCDE, DndCDE+DTT, and DndCDE+Dithionite. (C) EPR spectra of 13K [3Fe-4S]<sup>+</sup>, 13K Fe<sup>3+</sup>, and 77K [2Fe-2S]<sup>+</sup> at various H<sub>2</sub>O<sub>2</sub> concentrations and reaction times.

**Fe-S cluster and H<sub>2</sub>O<sub>2</sub> decomposition.**

(22)

(27)

(28, 29)

(29)

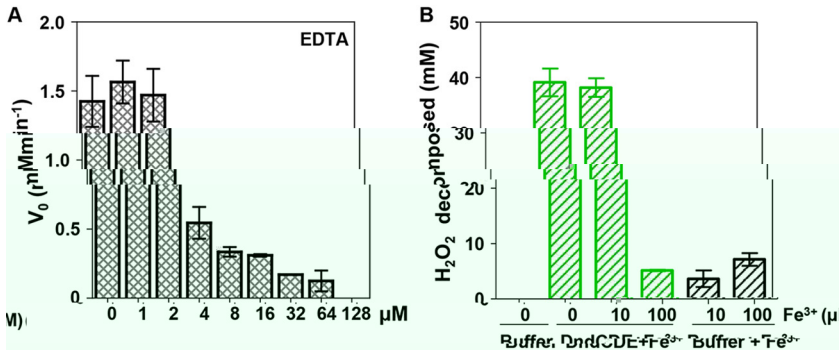


FIG 5 (A) V<sub>0</sub> (nmol/min) vs EDTA (μM) (n = 3).

g 2.035 2.001, (0.4A, ...). g 2.032 2.001 (0.4A, ...), 4 -4 + (27, 30). g 77, ( ... ), 13, g 2.029 2.002 (0.4, ...). A (0.4, ...). 3 -4 + (29) 3 -4 +. ( ... ). 4 -4 + 30 (10) (0.4, ...). 30 3+ g 4.3, ( ... ). 9.5 (0.4, ...). A 2 -2 + 30 (0.4, ...).

**DndCDE H<sub>2</sub>O<sub>2</sub> decomposition activity does not depend on ferric ion.**

3+ 3+ (31). A (32, 33). A ( ... )/ (34). A 0.5A. A 128 μ A, 2 -2 + 10 μ 100 μ

Downloaded from <http://aem.asm.org/> on February 25, 2021 at Shanghai Jiao Tong University

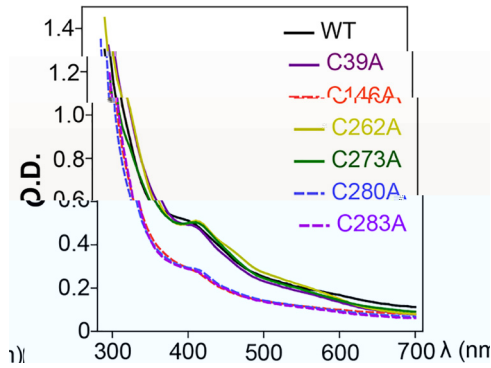
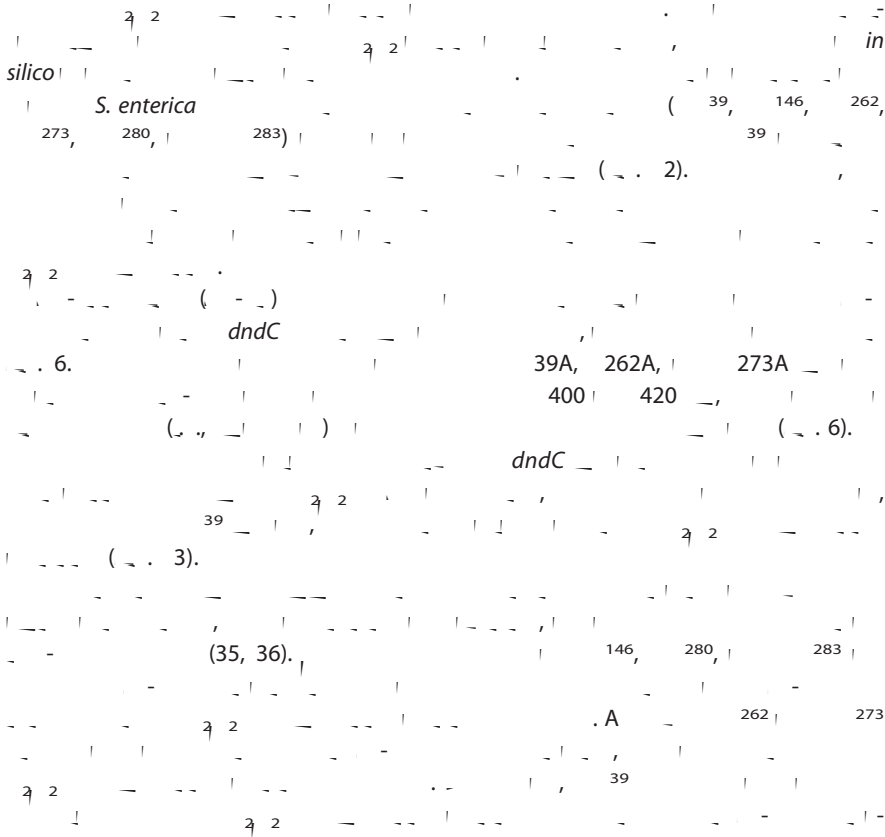


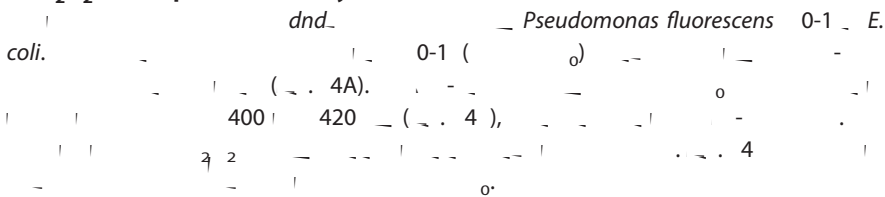
FIG 6

( $\lambda_{400}$ )  
130  $\mu$ m

**Conserved cysteine residues in DndC participate in H<sub>2</sub>O<sub>2</sub> decomposition.**



**H<sub>2</sub>O<sub>2</sub> decomposition activity of DndCDE from Pseudomonas fluorescens Pf0-1.**



**DISCUSSION**

*E. coli* *S. enterica*, 1,400  
(5), 3.4  
A  
(16)  
*Salmonella enterica*,  
(37, 38)  
( ) (39)  
A  
( .1).  
(6, 40),  
*dndCDE*  
(17), A  
A  
*Pseudomonas fluorescens* 0-1 ( . 4A  
) *S. enterica*, 2 ( )  
(41).  
*S. enterica*



**TABLE 1**

Strain or plasmid	Description	Reference or source
<i>Salmonella enterica</i> 87	<i>dndBCDE</i>	60
<i>Escherichia coli</i> 10	<i>mcrA</i> ( <i>mrr-hsdRMS-mcrBC</i> ),	
<i>Escherichia coli</i> 21(3)	<i>E. coli</i> 3, λ	7 A ;
-28 (+)	322	
-15	322	
	-28 A	52
	A	
	<i>dndE</i>	52
	<i>dndD</i>	52
	<i>dndC</i>	52
	-28 A	52
	-15 <i>iscS</i>	
- 39A	39A	
- 146A	146A	
- 262A	262A	
- 273A	273A	
- 280A	280A	
- 283A	283A	

(6).

1,400

10-

**MATERIALS AND METHODS**

**Bacterial strains, plasmids, and culture conditions.**

39A, 146A, 262A, 273A, 280A, 283A (52). *dndC*  
 (2) *E. coli* 3- *dndC* 19- 5'  
*dndC* 5'  
 (A, A) 4

**TABLE 2**

Oligonucleotide function and name	Sequence (5'→3')
39A-	AA A A AA AA AA
39A-A	A A AAA AA A AA A
146A-	A A A AAA AA
146A-A	A A AAA A
262A-	A A A A AAA A
262A-A	A A A AA A A
273A-	AA A A
273A-A	A A A A A A A
280A-	A A A A A AAA A
280A-A	A A AAA A A AA A
283A-	A A A A AAA A AAA A
283A-A	A A A A AAA A A
24 - A/ ( )	AA
24 - A/ ( )	AAA A A AA A
24 - sA/ s ( )	sAA
24 - sA/ s ( )	AAA A A s AA A

(A, A).  
 1% A A (1 A);  
 ).  
*E. coli* 10 (53).  
*dndC*  
 A (A) *E. coli* 21(3)/  
 (A, A, A).  
 (10 / , 5 / )  
 , 10 / ) 50 μ<sup>-1</sup>  
*E. coli* 1  
 50 μ<sup>-1</sup> 37 250  
 600 ( 600) 0.6 A  
 0.1  
 24 16 A 6,000 × g  
 20 -80

**Protein expression and purification. (i) N-terminal His-tagged cysteine desulfurase, IscS.**

(52) 1. 50  
 (20 8.0, 150 5% ) 50  
 ( A) 100 6.4  
 10 2- 4- ( 500:  
 ). 18,000 × g 20  
 A), 10 ( A ) A,  
 (20 8.0, 150 , 40  
 5% ). 5 2 (20  
 8.0, 150 , 500 5% ), 2.5  
 -10 ( A, A) 3.5 (20  
 8.0, 150 , 5% ) (54).

**(ii) C-terminal His-tagged Dnd protein complex DndCDE or DndCDE<sub>PFO</sub>.**

( ) 5.  
 ) 25 (20 8.0, 150 5%  
 ) 50- ( ).  
 A 1- ( ;  
 ), ( ) 1 (20  
 8.0, 25 5% ) 2 (20  
 8.0, 150 5% ). 5  
 (20 8.0, 500 5% ),  
 A ( )  
 200 10/30 ( ) 2.  
 1- 280  
 (λ<sub>280</sub>). (3 )  
 0.5 8,000 × 4 2 (A  
 ). (20  
 8.0, 150 , 5% ) 6 4  
 (54).

**(iii) Gradient native gel electrophoresis detection of both native and cross-linked DndCDE.**

( A, A) 20 (55).  
 4% 20%  
 (56). 4% 20%  
 1.2 ( )  
 49.5%;  
 3% , 5 3× (75 57.6  
 ), 100 μ 10% ( / ) (A ), 10 μ  
 ( ), 8.7  
 (49.5% , 3% ; , 5 3× (75  
 57.6 ), 3 , 75 μ 10% ( / ) A , 7.5 μ 918 μ  
 20% 4%  
 (A600457-0500;

**(iv) In vitro anaerobic enzymatic formation of active Fe-S cluster Dnd protein complex, DndCDE-FeS.**

( A) (23).  
 20 μ 30 1  
 α,α'- 2 -10  
 ( ) 3.5

900  $\mu$  0.5- 8,000  $\times g$   
 4 2 (A) (20  $\mu$ ) 20  $\mu$   
 7.5 0.5 ( ) 0.5  
 (20 8.0, 150 5%) 1 15,000  $\times g$   
 10 -10 ( -20 -10;  
 A, A) 4 0.5- ( -20 -10;

**UV-Vis and EPR analysis of DndCDE and DndCDE-FeS.**

( 2; A) 5  
 200- $\mu$  130  $\mu$ , 20 (20  
 8.0, 150 5%) 96- (3599; A) (20  
 $\lambda_{290}$   $\lambda_{700}$   
 ( )  
 130  $\mu$  (20 8.0, 150 5%  
 ) (2 ) 10/12  
 ( ) 910  
 A ( )  
 , 9.387 ; , 5  
 ; , 2 ; , 100 13 77  
 (130  $\mu$  ) 12  $\mu$  5 2 1,200  $\mu$   
 (200  $\mu$ ) 60  $\mu$  2.5 8.0  
 0.5 , 1 , 2 , 5 , 10

**Fenton reaction and HPLC-MS detection of phosphorothioate DNA.**

100- $\mu$   
 25  $\mu$  A ( 100 ) 100  $\mu$  2 A  
 1 2 ( 100 ) (31).  
 30 10  $\mu$   
 18 A 1100 / ( / )  
 0.1% 0.1% ( A 0.1%  
 30 0.1% 1% 13% 35.5 , 13% 30%  
 20 , 1% 10  $\lambda_{254,4}$   
 325 , 10 3,100  $m/z$  597 ( A), 565 ( A),  
 581 ( A)

**EMSAs.**

A ( A ) 2;  
 ( A ) / ( A ) 5' 6'  
 A/ A 500- $\mu$   
 50  $\mu$  400  $\mu$  , 100  $\mu$  5 $\times$   
 (150 8.0, 50 240 ) 300  $\mu$   
 100 10 A  
 20- $\mu$  A 2  $\mu$  5' A-1 (4  $\mu$ ), 2  $\mu$   
 4  $\mu$  (4  $\mu$ ) , 2  $\mu$  (100  
 8.0, 1 , 1 , 0.1 -1 A, 50% )  
 10  $\mu$  6.67  $\mu$   
 40 , 10  $\mu$  A 3.96% (49.5%  
 3% , 1 A) 0.5 $\times$  (44.5  
 44.5 , 1 A) 100 1 A  
 3000 ( )

**Measurement of DndCDE and DndCDE-FeS H<sub>2</sub>O<sub>2</sub> decomposition activities. (i) Colorimetric assay.**  
 100- $\mu$  20  
 8.0, 150 , 125 5% 40 2  
 1.67  $\mu$  (0.25 -<sup>-1</sup>) 25  
 20 5-  
 1,000- (20 8.0, 150 5%)  
 10 200  $\mu$   
 ( A ; ) (18, 57),  
 30

$\lambda_{595}$   $2^+$ ,  $10 \mu$   $100 \mu$   $2$   $2^+$   
 A  
 (ii) Bubble test. (26)

-100  $10$   $1$   $10 \mu$   $3$   $2^+$   
 20  $8.0$   $150$   $125$   $5\%$   $1\%$   $-100$ .  
 (26)

(iii) UV absorption.  
 $96$   $($   $)$   $2$   $($   $)$   
 $\lambda_{240}$   $($   $)$   $\lambda_{240}$  (58).  
 $0$ ,  $15$ ,  $20$ ,  $24$ ,  $30$ ,  $40$ ,  $48$ ,  $60$ ,  $80$ ,  $96$ ,  $120$ ,  $160$ ,  $200$ ,  $240$ ,  
 $(100\text{-}\mu$   $)$   $20$   $8.0$ ,  $150$   
 $125$   $5\%$   $1.67 \mu$   $A$   
 $3$   $2$   
 $1$   
 $5$   $($   $)$   $A$ ,  $A$ .  
 $A$ ,  $1 \mu$ ,  $2 \mu$ ,  $4 \mu$ ,  $8 \mu$ ,  $16 \mu$ ,  $32 \mu$ ,  $64 \mu$ ,  $128 \mu$   $A$

(iv) O<sub>2</sub> liberation rate measurement (pO<sub>2</sub> oxygen electrode).  
 $(59)$ .  $(2$   $)$   $50$   $2^+$   
 $1.67 \mu$   $125$   $($   $8.0$   $)$   $2$   
 $($   $)$

**SUPPLEMENTAL MATERIAL**

[:// /10.1128/A .00104-19](https://doi.org/10.1128/A.00104-19).  
 SUPPLEMENTAL FILE 1,  $0.2$

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$A$   
 $($   $)$   
 $(973$   
 $2015$   $554203$ )  $($   $31470830$ ,  
 $21661140002$ ,  $91753123$ ).

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