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Phosphorothioated DNA Is Shielded from Oxidative Damage

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ABSTRACT A A ( ) A *Salmonella enterica* (2 2) *Escherichia coli* ( ) *S. enterica.* A. A. ( ) 2 2 V 10.58 ± 0.90 $^{-1}$ $K_{0.5}$ 31.03 2.419 ± 0.59 2 2 ( ) *Pseudomonas fluorescens* 0-1. A 2 2

IMPORTANCE A A A A A 2 2 *E. coli* 2 2 A. 2 2

KEYWORDS A A 2 2

D A A A A A A

Citation Pu T, Liang J, Mei Z, Yang Y, Wang J, Zhang W, Liang W-J, Zhou X, Deng Z, Wang Z. 2019. Phosphorothioated DNA is shielded from oxidative damage. Appl Environ Microbiol 85:e00104-19. https://doi.org/10.1128/AEM.00104-19.

Editor M. Julia Pettinari, University of Buenos Aires

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Received 13 January 2019
Accepted 5 February 2019
Accepted manuscript posted online 8 February 2019
Published 4 2 19

A . A (1). A , A (2 4).

A (5, 6). *Clostridium difficile,* 60% (7). *Leptospira* A (8), A 50% *Mycobacterium abscessus* (9, 10).

(5, 6), *Escherichia coli* 7A *Salmonella enterica* 87, 10^4 ( ) (5). 10^3 *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. (1) $^{2+}$ (100 μ) ( ) . 1 A ( ) A (*m/z* 581) A (*m/z* 565) (15). A (13), A 1 100 μ .

Preferential complexation of DndCDE to PT DNA. A . *E. coli* ( ) (17) 1655 (17). A *in vivo* *dndCDE* *S. enterica* 87 *E. coli.*

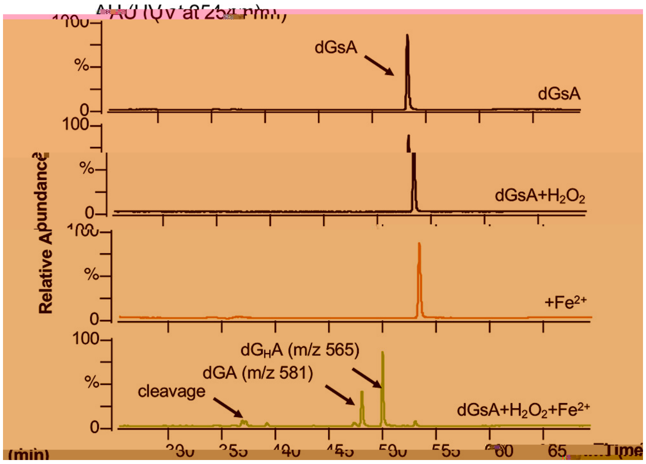

FIG 1 A 1 $_2$ 100 μ ($_4$)$_2$ ($_4$)$_2$. A

( ) (. 2A,).

440 880 (. 2A, 1).

440 880 (. 2A, 2).

A

(A). . 2

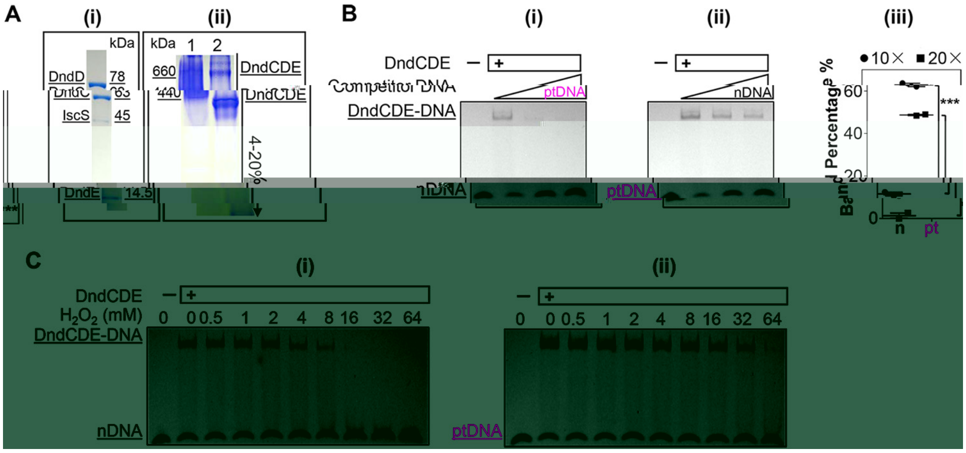

FIG 2 A. (A) A A. A, 200 A ( ). (/ /) ( ). 4% 20%. 1, 2 μ 2, 1 20 (1 μ) . ( ) A A. A 3.96% 0.5× 3.38 μ 0.4 μ A. A 3000 (., ,). A A A ( ) A ( ) A A A ( ). A A ( ). 10- 20- A ***, P < 0.01, t . ( ) A $_2$ A ( ) A ( ). A, A.

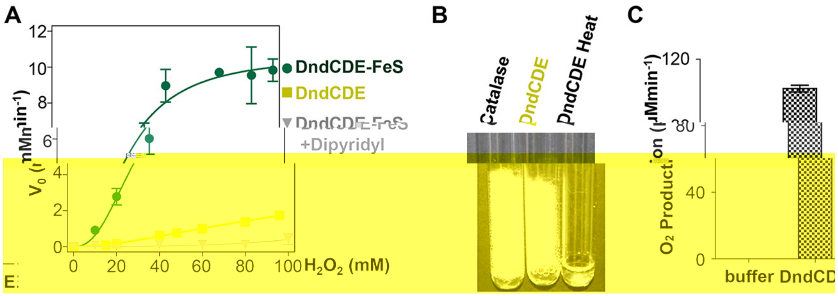

FIG 3 . (A) 1.67 μ α,α- (n = 3). ( ) 10 μ (125) 1% -100. , 600 (1896; , A) . ( ) 1.67 μ 50 (n = 3).

A, A A H_2O_2 A (. 2 ,), 16 H_2O_2 A . A , 64 (. 2 ,). 32 H_2O_2. A A .

DndCDE-FeS actively decomposes H_2O_2. (20, 21). / (22). (23), . , H_2O_2 . , (-) , H_2O_2 (. 3A), . α,α′- , (24, 25), . V 10.58 ± 0.90 $^{-1}$, H_2O_2 , $K_{0.5}$, 31.03 (. 3A) 2.419 ± 0.59. , H_2O_2 V 2.09 $^{-1}$ H_2O_2 $K_{0.5}$ 58.59 . 2.93 (. 3A). H_2O_2 (26). , H_2O_2 (. 3). H_2O_2 (. 3). H_2O_2 .

H_2O_2 decomposition requires an intact DndCDE. H_2O_2 , , , , , (. 1A).

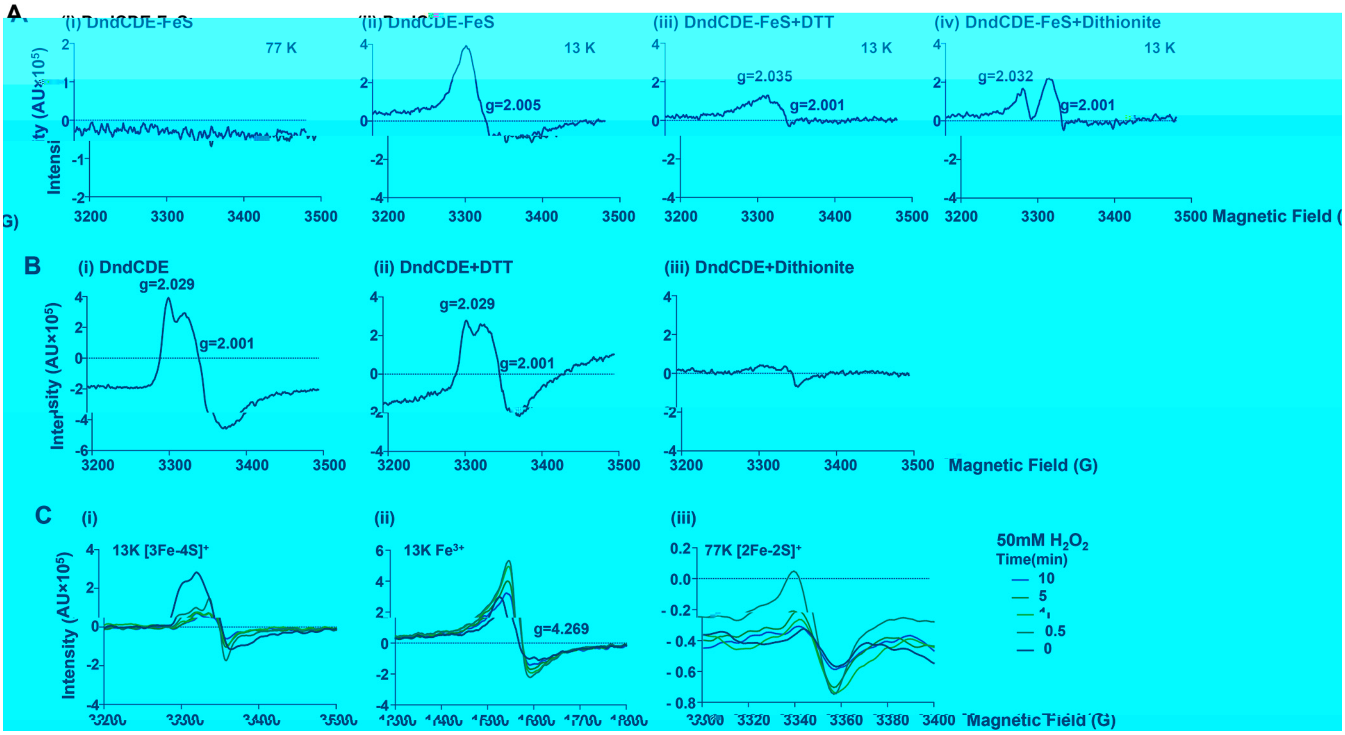

FIG 4 10/12 77 2 -2 13 4 -4 3 -4 . 130 μ . 3 -4 4 -4 2 ( ) . (A) : ( ), 2 ( ), 2 ( ). ( ) 13 : ( ), 2 ( ), 2 ( ). ( ) $_2$ $_2$. (.) , 50 $_2$ $_2$ 130 μ . 0, 0.5, 1, 5, 10 , .

$_2$ $_2$. A , (. 1). . A . 1 , $_2$ $_2$, V $K_{0.5}$ 1.56 $^{-1}$ 35.5 , .

Fe-S cluster and H_2O_2 decomposition. (-) (22). 2, 3, 4 2 4 , / 2 -2 , 3 -4 , 4 -4 . $^{2+}$ / $^{3+}$. *In vivo*, - 2 -2 4 -4 (22), . ( ) 77 13 2 -2 4 -4 (27). 77 , *g* (. 4A,), 13 *g* 2.005 , 4 -4 (. 4A,) (27). 4 -4 , (28, 29). 4 -4 $^{3+}$ 4 -4 $^{2+}$, 4 -4 $^{2+}$ 4 -4 $^{+}$ (29) 4 -4 $^{3+}$

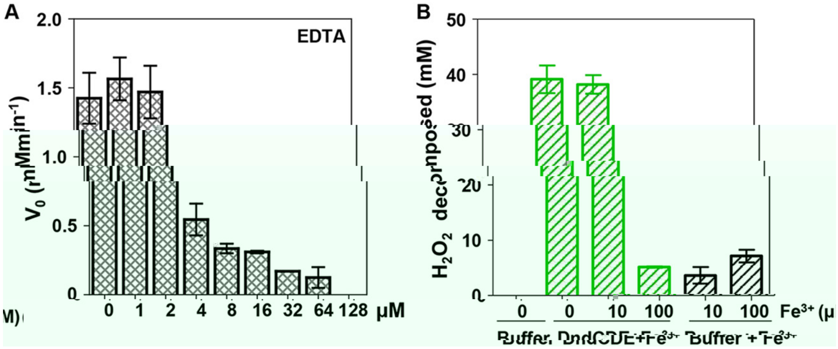

FIG 5 A $_2$ $_2$. (A) (V_0) $_2$ $_2$ A. ( ) $_2$ $_2$ (*n* = 3).

, *g* 2.035 2.001, (. 4A,). *g* 2.032 2.001 (. 4A,), 4 -4 $^+$ (27, 30).

, *g* 77 ( ), 13 , *g* 2.029 2.002 (. 4 ,). A (. 4 ,). , 3 -4 $^+$ (29) 3 -4 ^{0}.

. 4 $_2$ $_2$, ( ). 4 -4 $^+$ $_2$ $_2$ 30 (10) (. 4 ,). $^{3+}$, *g* 4.3, 30 9.5 (. 4 ,). A 2 -2 $^+$ 30 5 (. 4 ,).

DndCDE H_2O_2 decomposition activity does not depend on ferric ion. $_2$ $_2$, $^{3+}$. $^{3+}$ $_2$ $_2$ (31). A (32, 33). , A A- ( )/ $_2$ $_2$ (34). A $_2$ $_2$, . 5A. $_2$ $_2$ A , 128 µ A, . $_2$ $_2$, . . . 5 10 µ , . , 100 µ , $_2$ $_2$. ,

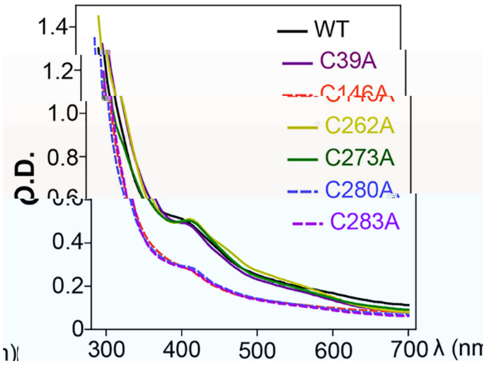

FIG 6 (λ_{400}).
130 μ.

$^{3+}$.

Conserved cysteine residues in DndC participate in H_2O_2 decomposition.

in *silico* *S. enterica* (^{39}, ^{146}, ^{262}, ^{273}, ^{280}, ^{283}) ^{39} (. 2).

() *dndC* . 6. 39A, 262A, 273A 400 420, (.,) (. 6). *dndC* ^{39} (. 3).

(35, 36). ^{146}, ^{280}, ^{283}. A ^{262} ^{273}, ^{39}.

H_2O_2 decomposition activity of DndCDE from *Pseudomonas fluorescens* Pf0-1.

dnd *Pseudomonas fluorescens* 0-1 *E. coli.* 0-1 ($_0$) (. 4A). $_0$ 400 420 (. 4), . 4 $_0$.

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 2 2 (. 4A). *S. enterica,* 2 2 ( ) (41). *S. enterica*

TABLE 1

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

(6). , 1,400 , 10- 2 2 .

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. 1, (52). , *dndC* 39A, 146A, 262A, 273A, 280A, 283A (2) . 19- 5′ *E. coli*, 3- *dndC* *dndC* 5′ . (, ., A, A) 4

TABLE 2 A [a]

Oligonucleotide function and name	Sequence (5′→3′)
39A-	AA A A AA AA
39A-A	A A AAA AA A AA A
146A-	A A A AAAA AA
146A-A	A AAAA A
262A-	A A A A AAA A
262A-A	A A A AA AA A A
273A-	AA A
273A-A	A A A A A A A
280A-	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 - - **s**A/ **s** ( )	**s**AA
24 - - **s**A/ **s** ( )	AAA A A **s** AA A

[a] .

A A (A , , A). 1% A (1 A ; .). E. coli 10 . (53). dndC A (A) E. coli 21(3)/ (A , A, A).

(10 / , 5 / , 10 /) 50 μ $^{-1}$, E. coli 1 50 μ $^{-1}$ 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 , 1- (A) (, A, A), 10 (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$_{\mathbf{Pf0}}$. (0) 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_{280}). (3) 0.5 8,000 × 4 2 (A , , ,). , 1 (20 8.0, 150 , 5%) 6 4 , (54).

(iii) Gradient native gel electrophoresis detection of both native and cross-linked DndCDE. 1 ( ) (, ., 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 . 6 (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 μ 0.5- 8,000 × g 4 2 (A ; , ,). (20 μ) 20 μ , 7.5 , 0.5 ($_4$)$_2$($_4$)$_2$, 0.5 (20 8.0, 150 , 5%) 1 , 4 . , 15,000 × g 10 , -10 ( ) 0.5- (-20 -10; , A, A) 4 . , - . .

UV-Vis and EPR analysis of DndCDE and DndCDE-FeS. (-) (2; , ., , A) 5 . 200-μ 130 μ, 20 , (20 - , 8.0, 150 , 5%) 96- (3599; , , A) λ_{290} λ_{700}. .

( ) , 130 μ (20 - , 8.0, 150 , 5%) . (2) (2) . 10/12 (., ., ,) 910 = (., ., ,) A ( ). : , 9.387 ; , 5 ; , 2 ; , 100 13 77 .

1,200 μ (130 μ) 12 μ 5 $_2$ 60 μ 2.5 , 8.0. (200 μ) 0.5 , 1 , 2 , 5 , 10 , .

Fenton reaction and HPLC-MS detection of phosphorothioate DNA. 100-μ 25 μ A (,) 100 μ $_2$. A 1 $_2$ (100) (31). 30 . 10 μ - $_{18}$ - (250 4.6 ; 5-μ ; ., ., ,) A 1100 / (/ -) . (A 0.1% 0.1%) 0.4 / 30 ., 1% 13% 35.5 , 13% 30% 20 , 1% 10 . . $\lambda_{254.4}$. 10 $^{-1}$, 30 / ^{2}, 325 , 3,100 . *m/z* 597 (A), 565 (A), 581 (A) .

EMSAs. A (A) 2; , (,) 5′ 6- (A) / , A/ . A 500-μ 50 μ 400 μ , 100 μ 5× (150 - , 8.0, 50 $_2$, 240), 300 μ . , 100 10 . A .

20-μ A 2 μ 5′ A- (4 μ), 2 μ 4 μ (4 μ) , 2 μ (100 - , 8.0, 1 , 1 , 0.1 $^{-1}$ A , 50%) 10 μ 6.67 μ - . 40 , 10 μ A 3.96% (49.5% 3% , - , ,) 0.5× (44.5 , 44.5 , 1 A). 100 1 A 3000 (., ,).

Measurement of DndCDE and DndCDE-FeS H_2O_2 decomposition activities. (i) Colorimetric assay. $_2$ 100-μ 20 - , 8.0, 150 , 125 , 5% , 40 $_2$ ( ). 1.67 μ (0.25 $^{-1}$) 25 20 . - 5- 1,000- (20 - , 8.0, 150 , 5%) 10 . 200 μ - (A ;) (18, 57), 30 ,

λ_{595}. $^{2+}$, 10 μ 100 μ $_2$ $_2$. A .

(ii) Bubble test. (26) $_2$ -100 10 . 1 10 μ , 3 $_2$, 20 , 8.0, 150 , 125 , 5% , 1% -100. $_2$. (26) ; .

(iii) UV absorption. $_2$ 96- ( ) 2 ( ) λ_{240} ($_2$ λ_{240}) (58). $_2$ 0, 15, 20, 24, 30, 40, 48, 60, 80, 96, 120, 160, 200, 240 , (100-μ) 20 , 8.0, 150 , 125 , 5% , 1.67 μ . A 3- 2 , $_2$. $_2$ $_2$. 1 , 5 (, A, A). A, 1 μ, 2 μ, 4 μ, 8 μ, 16 μ, 32 μ, 64 μ, 128 μ A $_2$.

(iv) O_2 liberation rate measurement (pO_2 oxygen electrode). $_2$. (59). ( ). (2-) 50 $_2$ $_2$ 1.67 μ 125 (8.0). $_2$ ($_2$) .

SUPPLEMENTAL MATERIAL

:// /10.1128/A .00104-19.

SUPPLEMENTAL FILE 1, , 0.2 .

ACKNOWLEDGMENTS

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

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51.