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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

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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 – ($_2$ $_2$- -)
(17 19). , -
. ,
A ( ) A.
- (-) -
A $_2$ $_2$.

RESULTS

Fenton reaction of PT DNA. $_2$ $_2$
- . ,
$_2$ $_2$ (1 .) $^{2+}$ (100 μ.)
. -
(-)
. . 1
A ( )
,
A (*m/z* 581) - A (*m/z* 565) (15).
- A (13),
. , A
1 . $_2$ $_2$ 100 μ. $_2$, .

Preferential complexation of DndCDE to PT DNA.
A .
E. coli –
($_2$ $_2$- -) $_2$ $_2$ (17)
- 1655 (17). A
in vivo $_2$ $_2$-
. , *dndCDE*
S. enterica 87 *E. coli*.

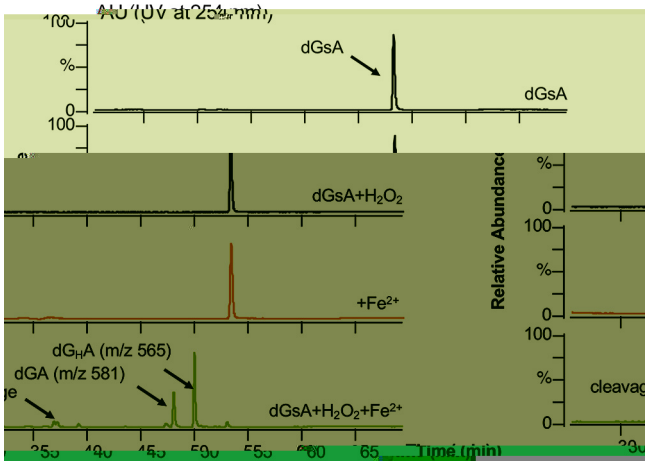

FIG 1 A 1 2 2 100 μ (4)2 (4)2. A , .

- : , , -
( ) (. 2A,).
, 440 880 (. 2A, , 1).
- , -
440 880 (. 2A, , 2).
A
(A). . 2 , .

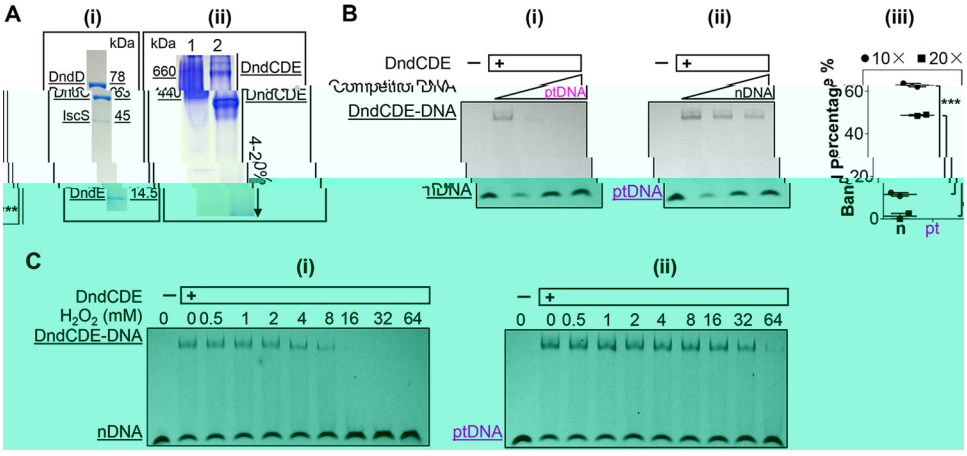

FIG 2 A. (A) A A . - A, , 200 , . - A ( ). (/ /) , . ( ). 4% 20%. 1, 2 μ . 2, - 1 20 . (1 μ) . ( ) A A. A 3.96% 0.5× . 3.38 μ . 0.4 μ . A. A 3000 (., ,). A A A ( ) A ( ) A , A A ( ). A ( ). 10- 20- A , . . ***, $P < 0.01$, *t* . ( ) A 2 2 A ( ) A ( ). A, A.

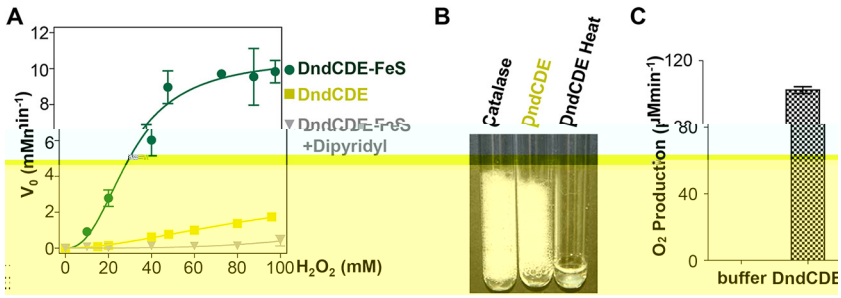

FIG 3 2 2 - . (A) 2 2 1.67 µ. 2 2 2 2 α,α- 2 2. (*n* = 3). ( ) 2 2 10 µ. - (125) 1% -100. - , 600 (1896; , A) . ( ) - 1.67 µ. 50 2 2 . (*n* = 3).

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

DndCDE-FeS actively decomposes H_2O_2. - - , (20, 21). , / (22). - (23), . , - 2 2 . , (-) . , 2 2 - (. 3A), - . - α,α′- , - (24, 25), . 2 2 - *V* 10.58 ± 0.90 $^{-1}$, - , $K_{0.5}$, 31.03 (. 3A) 2.419 ± 0.59. , 2 2 *V* 2.09 $^{-1}$ 2 2 $K_{0.5}$ 58.59 . . 2.93 (. 3A). 2 2 (26). , 2 2 (. 3). 2 2 - (. 3). 2 2 .

H_2O_2 decomposition requires an intact DndCDE. 2 2 , , , , , , (. 1A).

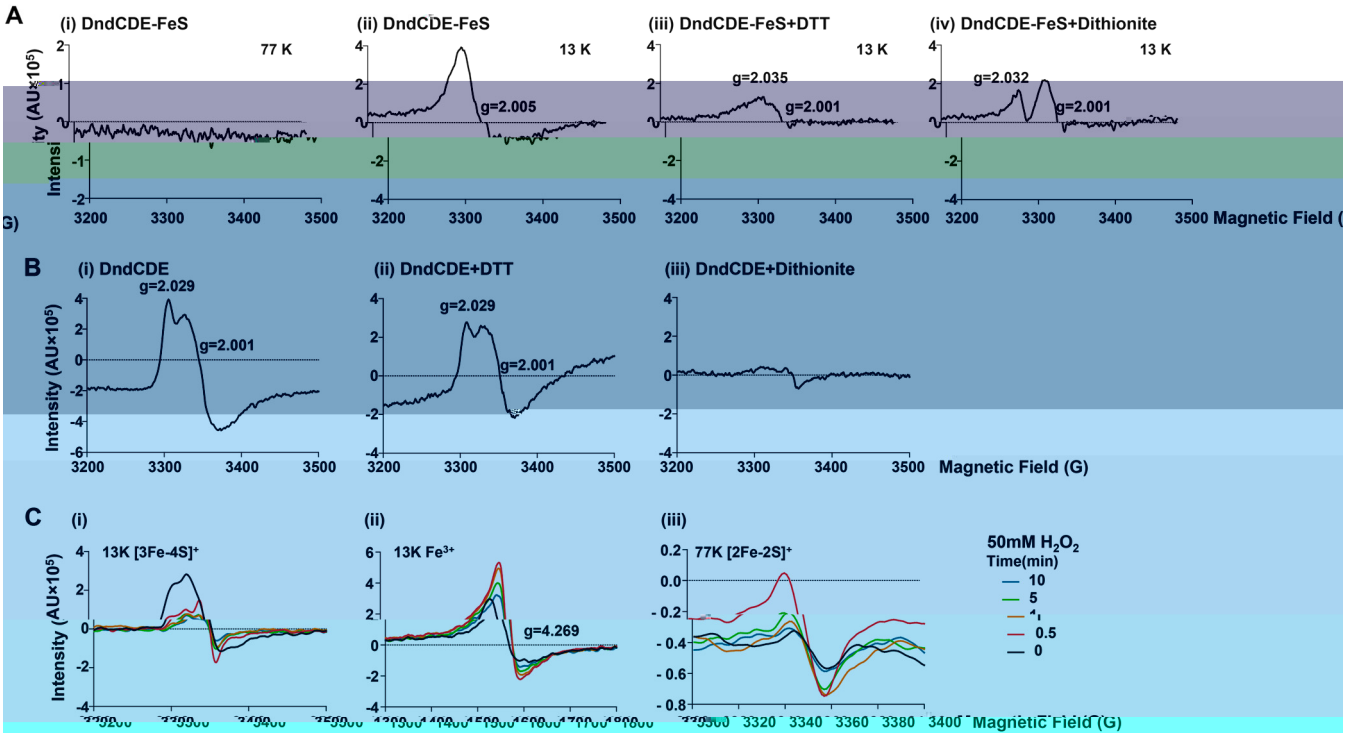

FIG 4 - . 10/12 .
77 2 -2 13 4 -4 3 -4 . 130 μ. -
. 3 -4 4 -4 2 ( ) . (A)
, : - - ( ), - 2 . ( ),
- 2 . ( ). ( ) - 13 :
( ), 2 ( ), 2 ( ). ( ) -
.) .
, 50 2 2 130 μ . 0, 0.5, 1, 5, 10 , .

. 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 $^{3+}$

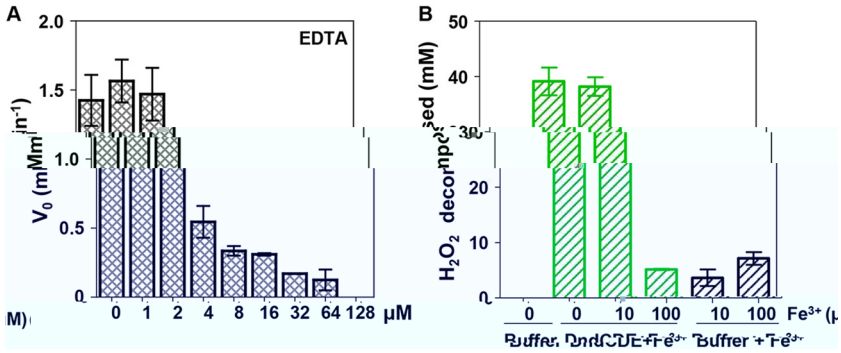

FIG 5 A . (A) (V_0) A. ( ) - (*n* = 3).

, , *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 ( ). 4 -4 + 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.

- - 3+ 3+ .

(31). A - (32, 33). , A A- ( )/ - (34). A , . 5A. A , 128 μ A, - , . 5 10 μ , . , 100 μ , ,

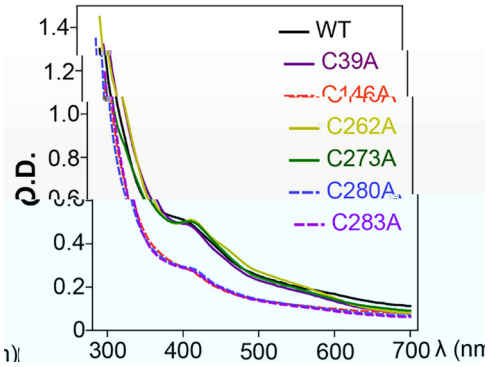

FIG 6

130 μ .

(λ$_{400}$).

2 2

3+.

Conserved cysteine residues in DndC participate in H_2O_2 decomposition.

2 2

2 2 , *in silico*

S. enterica (39, 146, 262, 273, 280, 283) 39

(. 2).

2 2

(-) -

dndC ,

. 6. 39A, 262A, 273A

400 420 ,

(.,) (. 6).

dndC

2 2 , ,

39 ,

2 2

(. 3).

, ,

- (35, 36). 146, 280, 283

-

2 2 . A 262 273

- ,

39

2 2 . ,

2 2 - -

.

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

dnd *Pseudomonas fluorescens* 0-1 *E. coli*.

0-1 (o) -

(. 4A). -

o

400 420 (. 4), - .

2 2 . . 4

o.

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 - ,	52
	A ,	
	, - *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$_{Pfo}$.
($_{O}$) 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 $_2$ $_2$ 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 $_2$ $_2$ (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. $_2$ $_2$ 100-μ 20 - ,
8.0, 150 , 125 , 5% , 40 $_2$ $_2$ ( ).
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. $_2$ $_2$ $_2$.
A . .

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

(iii) UV absorption. $_2$ $_2$ -
96- - ( ) 2. (
, .,) λ_{240} ($_2$ $_2$ λ_{240}) (58). -
$_2$ $_2$ 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
$_2$ $_2$. 3- 2. ,
$_2$ $_2$
$_2$ $_2$. -
, - 1. , .
. $_2$ $_2$
A 5 (, ., A, A).
A, 1 μ. , 2 μ. , 4 μ. , 8 μ. , 16 μ. , 32 μ. , 64 μ. , 128 μ. A
$_2$ $_2$.

(iv) O_2 liberation rate measurement (pO_2 oxygen electrode). -
$_2$ $_2$. (59). ($_2$ $_2$
). (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 , ,) . -. . (973 , 2015 554203) (31470830, 21661140002, 91753123).

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A *Mycobac-
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