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B cells Using Calcium Signaling for Specific and Rapid Detection of O157:H7

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A rapid and sensitive detection technology is highly desirable for specific detection of O157:H7, one of the leading bacterial pathogens causing foodborne illness. In this study, we reported the rapid detection of O157:H7 by using calcium signaling of the B cell upon cellular membrane anchors anti-O157:H7 IgM. The binding of O157:H7 to the IgM on B cell surface activates the B cell receptor (BCR)-induced Ca^{2+} signaling pathway and results in the release of Ca^{2+} within seconds. The elevated intracellular Ca^{2+} triggers Fura-2, a fluorescent Ca^{2+} indicator, for reporting the presence of pathogens. The Fura-2 is transferred to B cells before detection. The study demonstrated that the developed B cell based biosensor was able to specifically detect O157:H7 at the low concentration within 10 min in pure culture samples. Finally, the B cell based biosensor was used for the detection of O157:H7 in ground beef samples. With its short detection time and high sensitivity at the low concentration of the target bacteria, this B cell biosensor shows promise in future application of the high throughput and rapid food detection, biosafety and environmental monitoring.

48 3,000 ,
78 [1–3]. I
U S [4]. I , *Cam l bac e jej ni*, *Li e ia m n c gene*, *Salm nella en e ica*, *E. c di* O157:H7 - *E. c di* (-O157 STEC), *Vib i* . [5]. *E. c di* O157:H7
,
, , , , , (38%)[6].
, -
C - (CBB) [7].
CBB [8,9], [10] [11–15].
. B , , , [16–24]. A , B
BCR (B C R) T [25]. M B
(P -2E9) *Li e ia*, O
Bacill [13,25,26]. R *e al.*[11] B

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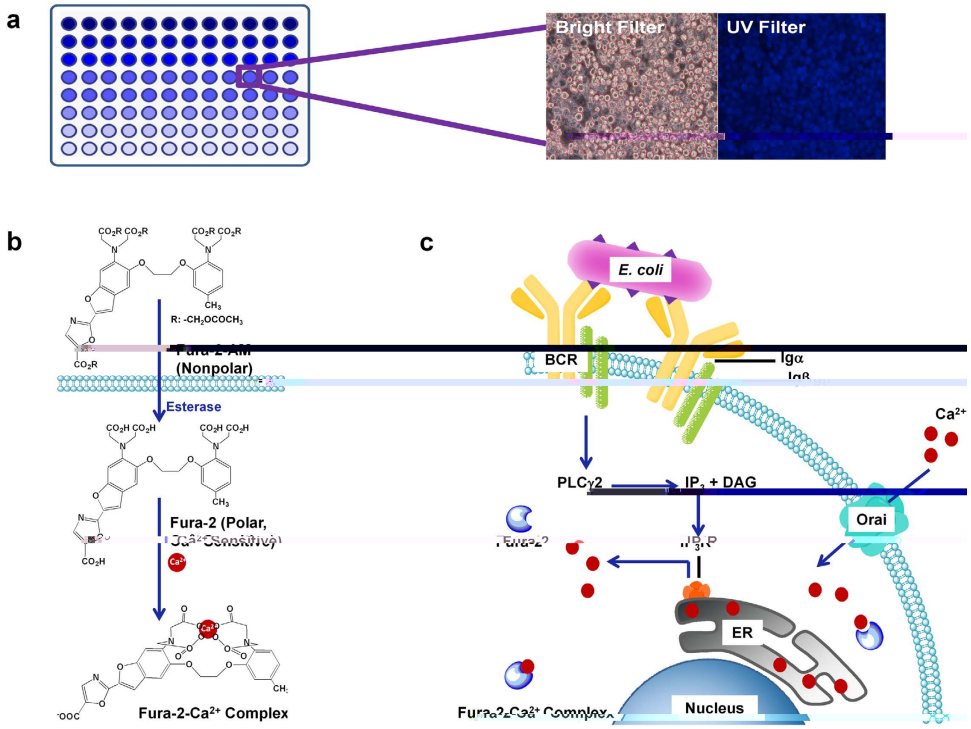

Figure 1. P B . (**a**). B . (**b**). S F -2 C 2+ . F -2 AM C 2+ . O , AM F -2- (C 2+ ,) , C 2+. (**c**). B . B , - B (BCR) , , C 2+ . F -2 340/380 .

5 [11]. B B B BCR C 2+ , B A - . F -2 (C 2+) [30,31]. (340:380) C 2+ [32,33]. I [34 36]. [37 39]. H , , F -2 B . I B F -2 B . *E. coli* O157:H7 , F -2 BCR- C 2+ B . C 2+- F -2 [31]. I C 2+. F -2 340 C 2+ 380 C 2+. I 340/380 C 2+[30]. *E. coli* O157:H7 .

Results

Principles of the B cell biosensor. C F -2 C 2+ B , C 2+- B *E. coli* O157:H7 . A F . 1 , F -2 B 96- , - (340:380) . F -2 (AM)

C 2+ . O
, AM F -2- (C 2+ ,
) C 2+ (F . 1).
B *E. c li* O157: H7.
BCR- C 2+ , B BCR- C 2+
[29]. A F . 1 , B
BCR[25]. BCR -
(I) I α I β. C BCR
[40]. BCR- PLC-γ2
4,5- (PIP_2)
1,4,5- (IP_3) (DAG). IP_3 IP_3
(IP_3R), (ER)
[41]. ER C 2+ , O ,
- (CRAC), CRAC . ,
C 2+ C 2+ ER C 2+ [29,42,43]. IP_3 -
, C 2+
, - [44 46].

Optimization of the B cell biosensor. B ,
C 2+ . MARC 29F8
(MA) LPS *E. c li* O157:H7[47]. U ,
B ELISA (E -
). ELISA S I . ELISA
(S F . S1) B
12 B . I -
(5 12) B B .
E. c li O157:H7 B (SEM).
R SEM F . 2 .
F -2 (*FR*) 1 . A 10^3 CFU/
L *E. c li* O157:H7 , 0 . A F . 2 ,
FR *E. c li* O157:H7, (HBSS
), . I ,
C 2+- (Ca_{min}) C 2+- (Ca_{ma}) . R
FR *C n l* Ca_{min} Ca_{ma} ,
.
F -2 *FR* *C n l* (F . 2) -
0 60 . F -2 ,
[32].
T C 2+ ,
C 2+ C 2+ -
B *E. c li* O157:H7 (10^1-10^9 CFU/ L). P -
, F . 2 . I NF (F -2), B F -2,
. NF6 (F -2, 10^6 / L)
NF5 (F -2, 10^5 / L) (< 0.0001). I NC (C 2+), B
F -2 C 2+ M 2+- . (> 0.05)
NF5 (F -2, 10^5 / L) NF6. I C (C 2+), B
F -2 C 2+ M 2+ . C6 (C 2+, 10^6 / L)
C5 (C 2+, 10^5 / L) ($= 0.0025$). S F -2
10^6 / L B , 10^6 / L B
. F -2 NF6
NC6 (C 2+, 10^6 / L) ($= 0.0324$) C6 (C 2+, 10^6 /
L) (< 0.0001). A C 2+ ($= 0.0006$) NC6
C 2+ M 2+- C6, C 2+
M 2+ .

Detection of O157:H7 in pure culture. B
E. c li O157:H7 10^1 10^5 CFU/ L .
HBSS *E. c li* O157:H7. C ,
E. c li O157:H7 10^2-10^5 CFU/ L. A
F . 3 -
10^1 10^3 CFU/ L. A , -
$= 0.0565$ $+ 0.6753$ ($R^2 = 0.96$). LPS
B (10^1-10^7 CFU/ L),
LPS
(F . 3). A , $= 0.03183$ $+ 0.6532$ ($R^2 = 0.83$).

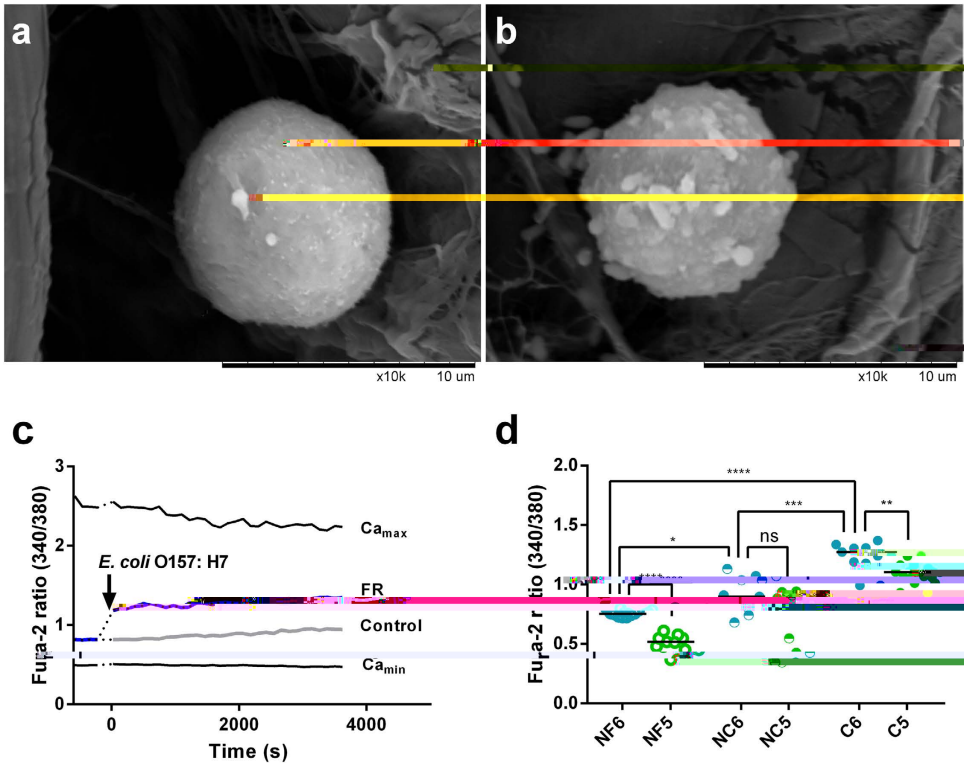

Figure 2. O B . (**a**). SEM B (×10000). (**b**). SEM B *E.c li* O157:H7 (×10000). (**c**). R F -2 340/380 . (**d**). C F -2 . D *E. c li* O157:H7 (10^1-10^9 CFU/ L) . B - - . (- , > 0.05, * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001, = 9) NF6: B (F -2, C $^{2+}$ M $^{2+}$ HBSS), 10^6 / L. NF5: B (F -2, C $^{2+}$ M $^{2+}$ HBSS), 10^5 / L. NC6: B (F -2, C $^{2+}$ M $^{2+}$- HBSS), 10^6 / L. NC5: B (F -2, C $^{2+}$ M $^{2+}$- HBSS), 10^5 / L. C6: B (F -2, C $^{2+}$ M $^{2+}$ HBSS), 10^6 / L. C5: B (F -2, C $^{2+}$ M $^{2+}$ HBSS), 10^5 / L.

S *E. c li* O157:H7 , B - , E *E. c li* 2 (O157 -O157) , , EHEC (-O157), . E 7 6 - (F . 3). - S T S5.

Li e ia m nc gene, *S.* T *V. a ahaem l ic* - , B (MA) LPS *E. c li* O157 N *Salm nella* . B (L , NH, USA) - I M (C : BE0087) B MARC 29F8. M 29F8 LPS 12- 16- D - LPS[47]. *L. m nc gene V. a ahaem l ic* G - G - A *S.* T , O- . N *Salm nella S. g de be g* - I ( ). *S.* T D (I: ,)[48].

R O C (ROC) (F . 3). F -2 *E. c li* O157:H7 ATCC 43888 - 30 , . ROC . ROC (AUR) 0.7319, 0.7690, 0.8484, 0.7817 0.7885, *E. c li* O157:H7 10^1, 10^2, 10^3,10^4 10^5 CFU/ L. A AUR *E. c li* O157:H7 0.7

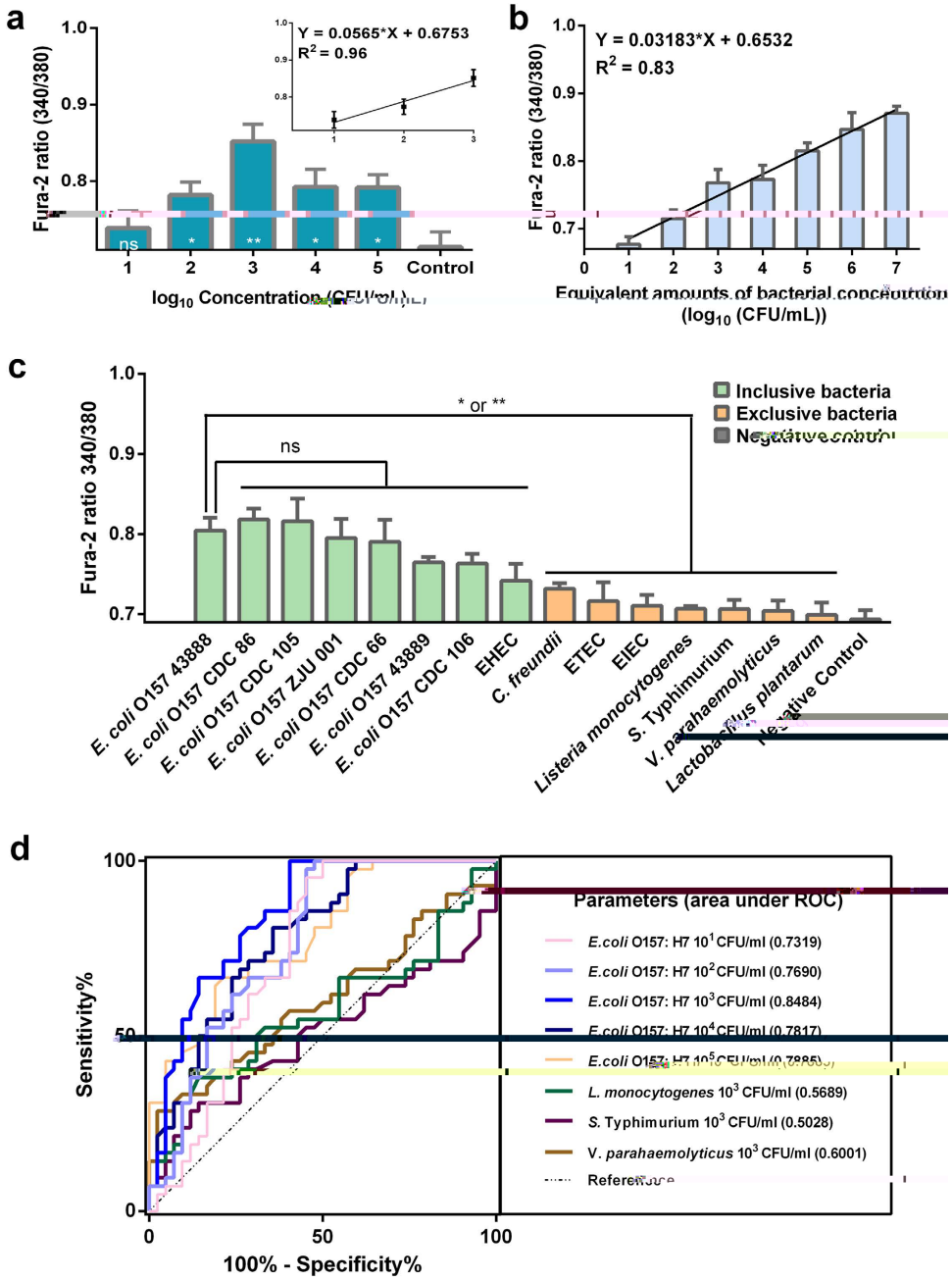

Figure 3. D *E. coli* O157:H7 . (**a**). P , F -2 , *E. coli* O157:H7 . I : L *E. coli* O157:H7 10^1 10^3 CFU/ L. A ± . . . - . (- , >0.05, * <0.05, ** <0.01, =4). (**b**). P , F -2 , LPS *E. coli* O157:H7 10^1 10^7 CFU/ L. A ± . . . - . (- , >0.05, * <0.05, ** <0.01, =3). (**c**). I B , - 10^3 CFU/ L. A ± . . . - . (- , >0.05, * <0.05, ** <0.01, =3). (**d**). ROC 30 .

0.7319 0.8484, B . I AUR 0.7817 0.7885 *E. coli* O157:H7 10^4 CFU/ L 10^5 CFU/ L, 10^3 CFU/ L *L. monocytogenes*, *S.* T , *V. parahaemolyticus* , AUR 0.5689, 0.5028 0.6001,

Detection of

I *E. c li* O157:H7 , B *E. c li* O157:H7 (10^1-10^3 CFU/ L). *E. c li* O157:H7 10^4 CFU/ L 10^5 CFU/ L, [illegible] *E. c li* B -[50], [illegible] BCR- C $^{2+}$. T [illegible] , LPS *E. c li* O157:H7 [illegible] B . A F . 3 , [illegible] LPS (10^1-10^7 CFU/ L). I [illegible] [illegible] B - T [51].

I *E. c li* O157:H7 , - B . O MA B O- LPS G - , EHEC (-O157) G - *L. m n c gene Lac bacill lan a m*, [illegible] I F . 3 , [illegible] - (-) - (S [illegible] T S5), , *E. c li* O157:H7 [illegible] [illegible] .

B ROC F . 3 , B [illegible] 10 10 30 . S *E. c li* O157:H7 10^1 CFU/ L, *E. c li* O157:H7 10^2-10^5 CFU/ L.

F , B 5.9×10^2 CFU/ L. A [illegible] 10 . I 10^2 10^4 CFU/ L, (SPR)[52], [illegible] - [illegible] (ELISA)[53], [54] [illegible] (QCM)[55]. *E. c li* O157:H7 7 CFU - (SPCE) 70 [56], 10 CFU/ L [illegible] - - [illegible] (LRSP-FS) 40 [57]. , 67 CFU/ L [illegible] - - (MNP) 8 [58], 500 CFU/ [illegible] CANAR ([illegible]) 5 [11]. I [illegible] [illegible] 30 μL , B [illegible] 18 *E. c li* O157:H7. I , B L. [illegible] , , .

F , B 8.6×10^2 CFU/ L (26 CFU 30 μL) *E. c li* O157:H7 . A [illegible] , [illegible] - [illegible] . I [illegible] [illegible] B . B [illegible] C $^{2+}$ F -2 C $^{2+}$ - B [illegible] *E. c li* O157:H7 . B [illegible] , . , [illegible] [illegible] , [illegible] F -2. T , [illegible] B , [illegible] .

I [illegible] B MARC 29 8 C $^{2+}$ - F -2 [illegible] . B[illegible] *E. c li* O157:H7 , [illegible] C $^{2+}$ [illegible] BCR C $^{2+}$. B [illegible] C $^{2+}$ F -2 [illegible] B . B 10^2 CFU/ L 10 . [illegible] B [illegible] *E. c li* O157:H7 [illegible] B [illegible] , - [illegible] [illegible] .

Methods

Reagents. D ' E ' (DMEM), D ' E ' (DMEM PR), - (FBS), H ' (HBSS), C $^{2+}$ M $^{2+}$- HBSS, MEM - (MEM NAA), 0.4% T B S , F -2/AM 0.5 M EDTA L T (C , CA, USA). A BD (S , MD, USA). G I M-HRP ( ), L (LPS) A D (A D S , USA), I B (G - , K) S B (S , C), A S (S . L , MO, USA). A - (DI) M D -8 (B , MA, USA).

B cell lines and culture conditions. B MARC 29F8 ATCC (A T C C , M , A; ATCC CRL-2508). B , B , I M. I DMEM 4 M L- 1.5 /L , 4.5 /L , 1 % MEM NAA 10% - FBS. 37 C 7% CO_2. L - - MARC 29F8 (5 10) 1:10 72 . MARC 29F8 DMEM 10% - FBS T-25 T-75 (F , O , USA) (72) . TC10 A C C (B -R , H , CA, USA) , .

Bacterial strains and culture conditions. B A T C (ATCC), C C I C C (CICC), C N C M C C (CMCC), C C D C P (P CDC) U (JU). S T S1 T S2.

Lac bacill lan a m MRS BHI 37 C, - *Li e ia m n c gene* 48 24 , - T S A (TSA). S I .

Scanning electron microscopy (SEM) imaging SEM (S I) H TM1000 (SEM) (H , J).

Preparation of a B cell biosensor. B B C $^{2+}$ - F -2, C $^{2+}$ F -2[33]. I , C $^{2+}$, - MARC 29F8 DMEM PR 37 C 5 . B HBSS DMEM (PR) . 4.5 F -2/AM 10^6 30 37 C 30 L HBSS . A 15 - F -2/AM, , 30 L HBSS C $^{2+}$ M $^{2+}$- HBSS .

Detection of O157:H7 in pure culture. B 30 μ F -2 MARC 29F8 B 96- , 30 μ . T , (340 380) F -2 MARC 29F8 0.1% T -100 4.5 M - (EDTA), F 340- 380- (F340 F380) 510 S H1 H M -M M (B T , , T, USA). D 120 . D 340/380 C $^{2+30}$. *E. c li* O157:H7 ATCC 43888 HBSS 6.2×10^1 6.2×10^9 5.9×10^1 5.9×10^5 , E LPS *E. c li* O157:H7 ATCC 43888 B . S I .

T 8 7 10^3 CFU/ L , (S T S1 T S2). *E. c li* O157:H7 ATCC 43888 HBSS F -2 B

, .

Detection of O157:H7 in ground beef. T B , - . , *E. coli* O157:H7 - . T - 225 HBSS F (L I ., Q , C) S 400 (S , N , UK) 1 . . O *E. coli* O157:H7 10^3, 10^4, 10^5 CFU/ L 9 , *E. coli* O157:H7 10^2, 10^3, 10^4 CFU/ L. *E. coli* O157:H7 HBSS . F -2 B .

Statistical analysis. D ± . . . ( ) - . G P P (G P , S D , CA). P -

Optimization of the B cell biosensor *E. coli* O157: H7 U - . < 0.05 .

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Author Contributions

L. R. . R. . L. . B.- K., S. J., K. . F. - . L. .

Additional Information

Supplementary information :// . . /

Competing financial interests: .

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