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

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Ling Wang[1,2], Ronghui Wang[2], Byung-Whi Kong[3], Sha Jin[4], Kaiming Ye[4], Weihuan Fang[5] & Yanbin Li[1,2]

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 li O157:H7 - *E. c li*
(-O157 STEC), *Vib i* . [5]. *E. c li* O157:H7
,
, , , ,
(38%)[6].
,
-
C - (CBB) [7].
CBB [8,9], [10] [11 15].
. B , ,
CBB , B , , T , [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

[1]College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China. [2]Department of Biological and Agricultural Engineering, University of Arkansas, Fayetteville, AR 72701, USA. [3]Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA. [4]Department of Biomedical Engineering, Binghamton University-SUNY, Binghamton, NY 13902, USA. [5]College of Animal Sciences, Zhejiang University, Hangzhou 310058, China. Correspondence and requests for materials should be addressed to Y.L. (email: yanbinli@zju.edu.cn)

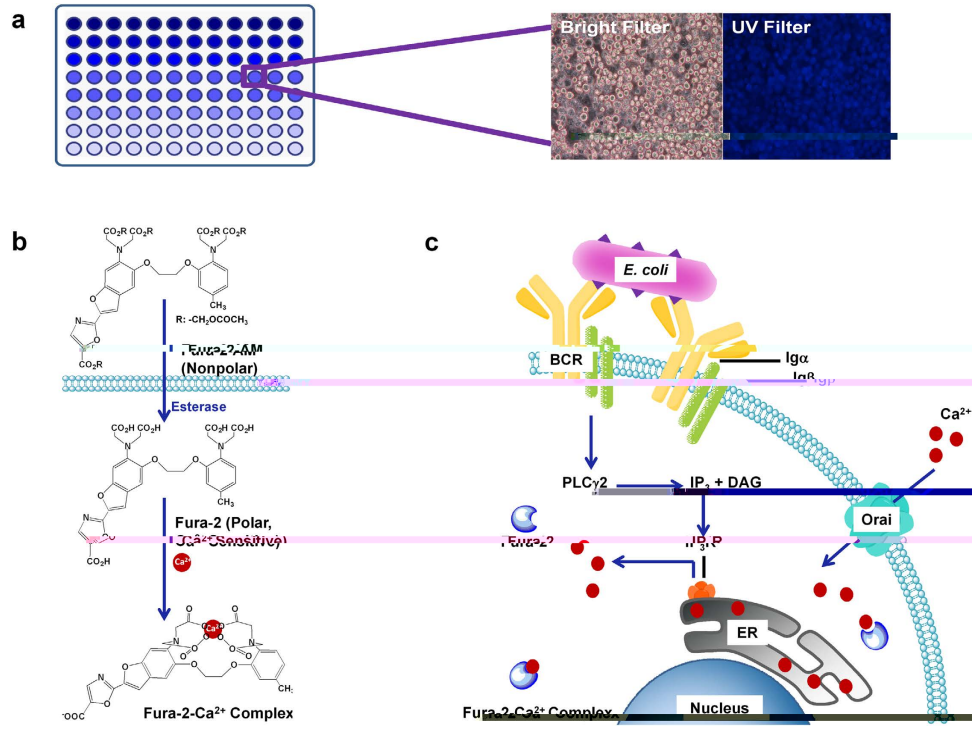

Figure 1. P B . (**a**). B B . (**b**). S F -2 C $^{2+}$. F -2 AM C $^{2+}$. O , AM F -2- (C $^{2+}$,) , C $^{2+}$. (**c**). B , - B (BCR) , C $^{2+}$. F -2 340/380 .

5 ^{11}. B BCR A . F -2 (C $^{2+}$) (340:380) C $^{2+}$ ^{32,33}. I $^{34\ 36}$. $^{37\ 39}$. H , F -2 B I B *E. c li* O157:H7 C $^{2+}$- , F -2 BCR- C $^{2+}$ B . C $^{2+}$- F -2 ^{31}. I F -2 C $^{2+}$. F -2 340 C $^{2+}$ 380 C $^{2+}$. I 340/380 C $^{2+30}$. *E. c li* O157:H7 .

E. c li O157:H7 500 CFU/ (<1) ^{27,28}, . O B BCR ^{29}. - ^{30,31}.

Results

Principles of the B cell biosensor. C F -2 C $^{2+}$, C $^{2+}$- B B , F -2 B 96- , *E. c li* O157:H7 . A F . 1 , F -2 B . F -2 (AM) (340:380)

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 α I β. C BCR [40]. BCR- PLC-γ2 4,5- (PIP$_2$) 1,4,5- (IP$_3$) (DAG). IP$_3$ IP$_3$ (IP$_3$R), (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_{max}) . R *FR* C n l Ca_{min} Ca_{max} , .
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 , $= 0.03183 + 0.6532$ ($R^2 = 0.83$).

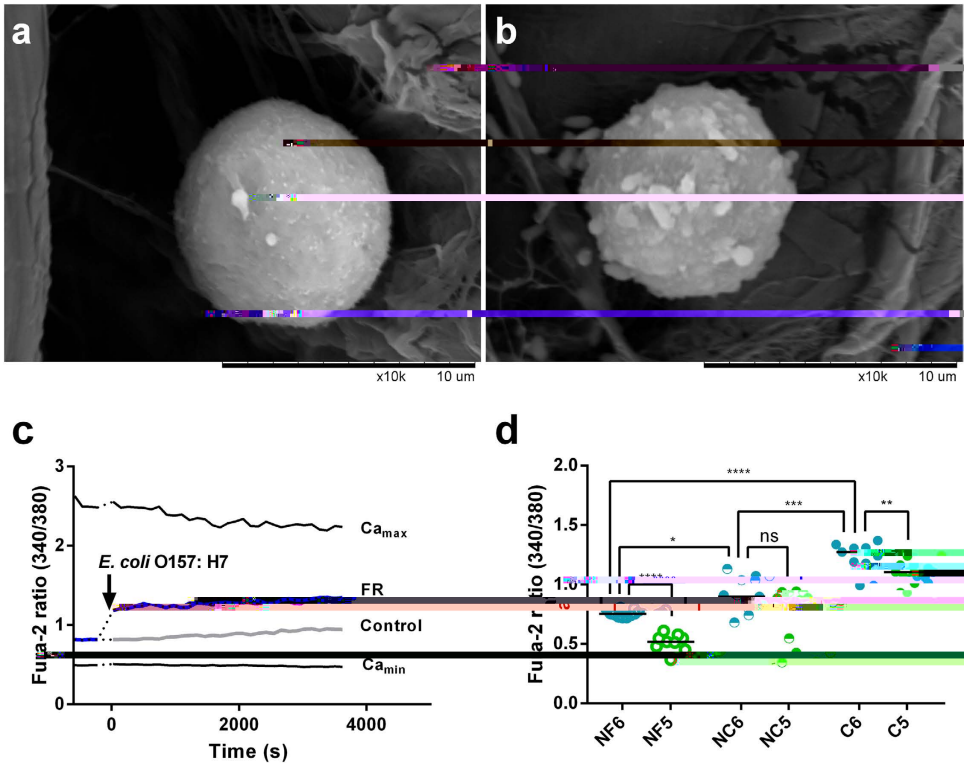

Figure 2. O (**a**). SEM B *E.c li* O157:H7 (×10000). (**b**). SEM B *E.c li* O157:H7 (×10000). (**c**). R F -2 340/380 . (**d**). C F -2 *E. c li* O157:H7 (10^1-10^9 CFU/ L) . B - . (- , > 0.05, * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001, $= 9$) NF6: B (F -2, C $^{2+}$ M $^{2+}$ HBSS), 10^6 / L. 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 n c 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 n c gene V. a ahaem l ic* G - G - A *S.* T , O- . I ( ). *S.* T D (I: 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

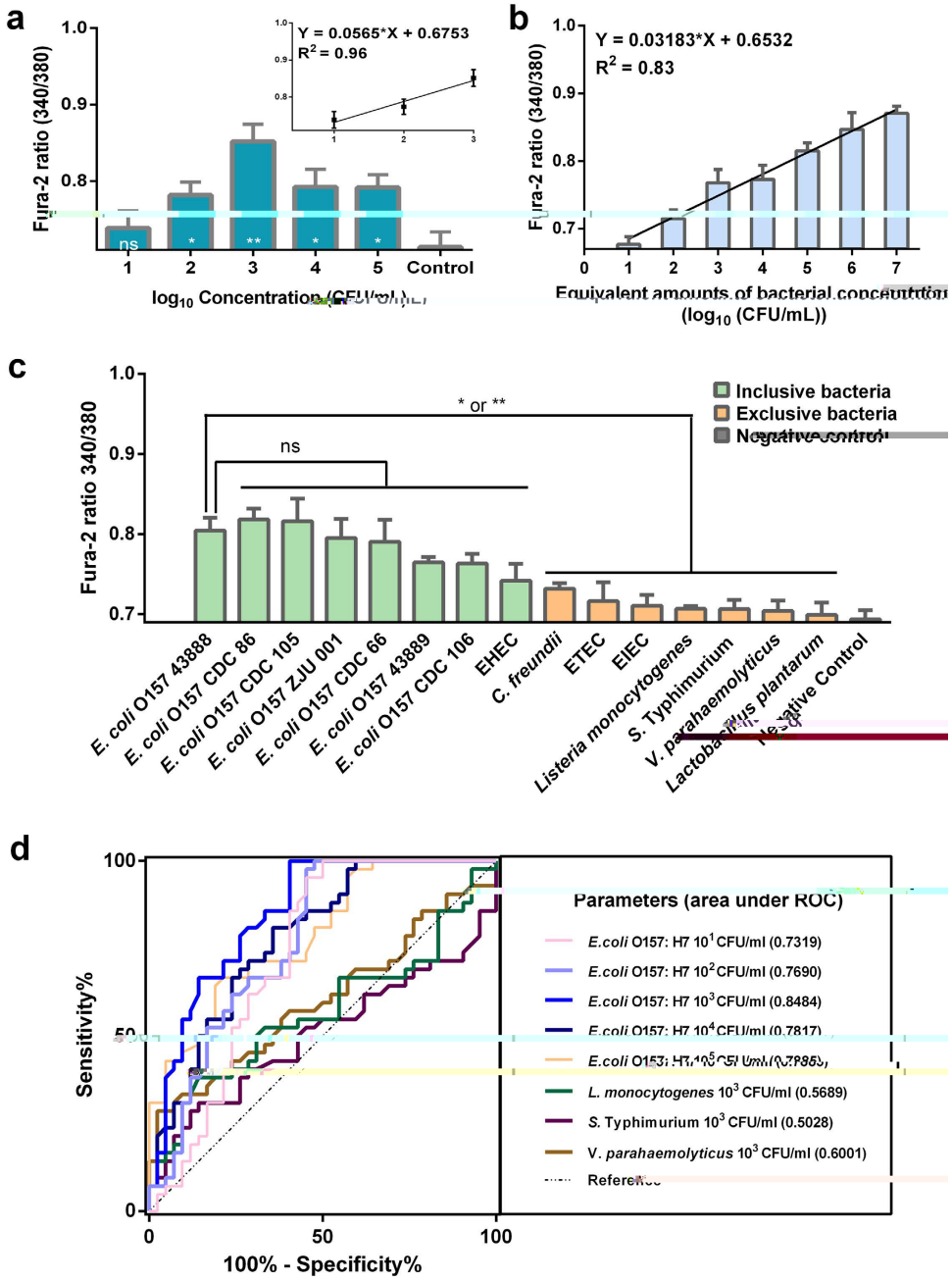

Figure 3. D *E. c li* O157:H7 . (**a**). P , F -2 , *E. c li* O157:H7 . I : L *E. c li* O157:H7 10^1 10^3 CFU/ L. A ± . . . - . (- , >0.05, * <0.05, ** <0.01, $=4$). (**b**). P , F -2 , LPS *E. c li* O157:H7 10^1 10^7 CFU/ L. A ± . . . - . (- , >0.05, * <0.05, ** <0.01, $=3$). (**c**). I B - 10^3 CFU/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

0.7319 0.8484, B . I AUR 0.7817 0.7885 *E. c li* O157:H7 10^4 CFU/ L 10^5 CFU/ L, 10^3 CFU/ L *L. m n c gene* , *S.* T , *V. a ahaem l ic* , 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, *E. c li* B [50], BCR- C $^{2+}$. T , LPS *E. c li* O157:H7 B . A F . 3 , LPS (10^1-10^7 CFU/ L). I - 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,* I F . 3 , - (-) - (S T S5), , *E. c li* O157:H7 .

B ROC F . 3 , B 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 10 . I 10^2 10^4 CFU/ L, (SPR)[52], - (ELISA)[53], [54] (QCM)[55]. *E. c li* O157:H7 7 CFU - (SPCE) 70 [56], 10 CFU/ L - - (LRSP-FS) 40 [57]. , 67 CFU/ L - (MNP) 8 [58], 500 CFU/ CANARD ( ) 5 [11]. I 30μL , B 18 *E. c li* O157:H7. I , L. , , .

F , B 8.6×10^2 CFU/ L (26 CFU 30μL) *E. c li* O157:H7 . A , . I B . B C $^{2+}$ F -2 C $^{2+}$ - B *E. c li* O157:H7 . B ' , . , , F -2. T , B , .

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

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 30μ F -2 MARC 29F8 B 96- , 30μ . 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. c li* O157:H7 - . T - 225 HBSS F (L I ., Q , C) S 400 (S , N , UK) 1 . . O *E. c li* O157:H7 10^3, 10^4, 10^5 CFU/ L 9 , *E. c li* O157:H7 10^2, 10^3, 10^4 CFU/ L. *E. c li* 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. c li* 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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