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The Patrol Yeast: A new biosensor armed with antibody-receptor chimera detecting a range of toxic substances associated with food poisoning

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ABSTRACT

Baker’s yeast is an attractive host with established safety and stability characteristics. Many yeast-based biosensors have been developed, but transmembrane signal transduction has not been used to detect membrane-impermeable substances using antigen-antibody interactions. Therefore, we created Patrol Yeast, a novel yeast-based immunosensor of various targets, particularly toxic substances in food. A membrane-based yeast two-hybrid system using split-ubiquitin was successfully used to detect practically important concentration ranges of caffeine and aflatoxins using separated variable regions of an antibody. Moreover, enterohemorrhagic Escherichia coli O157 was detected using a specific single-chain antibody, in which Zymolyase was added to partially destroy the cell wall. The incorporation of secreted Cypridina luciferase reporter further simplified the signal detection procedures without cell lysis. The methodology is more cost-effective and faster than using mammalian cells. The ability to detect various targets renders Patrol Yeast a valuable tool for ensuring food and beverage safety and addressing other environmental and technological issues.

1. Introduction

The contamination of foods with toxins such as mycotoxins, as well as viruses and bacteria affects human health and wellbeing. Among mycotoxins produced by fungi in deteriorated foods, aflatoxins produced by Aspergillus flavus are highly carcinogenic, and can cause liver cancer and even fatal incidents (Henry et al., 2002). Aflatoxin B1 or B2 produced by A. flavus is usually found in crops, such as corn and peanuts. They are quite stable and can easily contaminate food that is then consumed by livestock such as cattle (Rushing and Selim, 2019). Livestock animals could then produce aflatoxin M1 through metabolic reactions, which often result in contaminated milk (Marchese et al., 2018). Other important food contaminants include enterohemorrhagic bacteria, such as Escherichia coli (E. coli) O111 and O157 that can contaminate raw foods such as vegetables and meat and cause bloody diarrhea and other fatal symptoms in humans and other animals (Gally and Stevens, 2017). Traditional methods for analyzing these food contaminants include real-time polymerase chain reaction (PCR) (Spano et al., 2005), gas chromatography-mass spectrometry (GC/MS), and liquid chromatography-tandem mass spectrometry (LC/MS/MS) (Sontag et al., 2019), which require expensive instruments and trained personnel. Although immunoassays such as enzyme-linked immunosorbent assay (ELISA) have relatively simple requirements (Engvall and Perlmann, 1972), they still involve a series of operations that require skilled personnel. Nanomaterial-based sensors (Goud et al., 2018; Hossain et al., 2021; Mahmud et al., 2022) can also detect toxins and pathogens, but their manufacturing process is relatively complicated. Therefore, a rapid, convenient, and affordable analytical method is needed to reduce

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risk of food poisoning, especially in developing countries.

Cell-based biosensors offer an alternative approach to identifying food contaminants, especially when an antibody is the recognition unit. Much effort has been directed towards using antibody-based transmembrane (TM) receptors expressed on the plasma membrane of mammalian cells (Manhas et al., 2022; Scheller et al., 2018; Ueda et al., 2000) as biosensors, and a similar system has been developed in E. coli (Chang et al., 2018, 2021). As far as we can ascertain, such a system has not been established for yeast cells, probably because they have thick and stable cell wall. Nonetheless, many yeast-based biosensors have been developed because these well-studied eukaryotic species can withstand harsher environments than bacteria or mammalian cells while being safe for humans (Jarque et al., 2016). Moreover, yeast native or recombinant reporter enzymes can produce fluorescent or luminescent reporter signals.

The拟议的Patrol Yeast biosensors are novel MbY2H systems to identify various substances from small molecules to microorganisms irrespective of cell wall or membrane-permeability, and lead to food poisoning (Fig. 1). Patrol Yeasts detected caffeine and aflatoxins using the split-ubiquitin system. The secreted Cypridina luciferase reporter enzyme can also be integrated into this system for easier and safer high-throughput screening. The Patrol Yeast system could serve as a less labor-intensive tool to safely and economically identify food contaminants.

2. Materials and methods

2.1. Materials

Restriction enzymes were obtained from New England Biolabs Inc. (Ipswich, MA, USA). Chemical reagents were supplied by Fujifilm Wako Pure Chemicals (Osaka, Japan) unless otherwise stated. The monoclonal anti-aflatoxin (AFx) antibody 5A7 was generated by ARK Resource Co. Ltd., Kumamoto, Japan.

2.2. Plasmid construction

The plasmids pEF-BOS-BGPVH-mEpOR and pEF-BOS-BGPV1-mEpOR are pEF-BOS (Mizushima and Nagata, 1990)-based mammalian expression vectors for Vh-EpOR and V1-EpOR, respectively. They have been created by replacing the Vh/V1 genes of hYHEL-10 in pME-Vh/V1-ER (Ueda et al., 2000) with those of KTM219 (Lim et al., 2007), respectively, and allow BGP-dependent growth of Ba/F3 Pro-B cells (unpublished). A Vh(BGP)-EpOR DNA fragment was amplified using the primers Not-VLbgg back and Sfl-EpOR HTM For from the plasmid pEF-BOS-BGPV1-mEpOR. The yeast SUC signal sequence was first amplified by annealing the Spe-Suc back and Suc_for primers. The resultant SUC sequence was fused with Vh(BGP)-EpOR by overlap extension PCR (OE-PCR) to obtain the Suc-Vh(BGP)-EpOR fragment. The Vh(BGP)-EpOR fragment was amplified from pEF-BOS-BGPV1-mEpOR template using the SUR-VH-S1/SUR-VH-S2/SUR-VH-S3/Spe-Suc back and Sfl-EpOR HTM For primers, then fused to the Suc sequence as described above, to obtain the SUC-Vh(BGP)-EpOR fragment. Suc-Vh(BGP)-EpOR and Suc-V1(BGP)-EpOR were then inserted into pPR3-C (Dualsystems Biotech AG., Schlieren, Switzerland) and linearized with the restriction enzymes Spel and SfiI to construct pPR-Vh(BGP)-EpOR and pPR-V1(BGP)-EpOR. The Suc-Vh(BGP)-EpOR and Suc-V1(BGP)-EpOR DNA fragments were amplified using the primers Xba-Suc back and Sfl-EpOR HTM For and inserted into a pBT3-C vector (Dualsystems Biotech AG.).

The Vh(BGP)-DNA fragment was amplified from pET-Vh(BGP)-GS-GUSvs-KW using the primers NotVHback and BamVHHfor (Su et al., 2019). Vh(BGP)-DNA was then inserted into pPR-Vh(BGP)-EpOR and linearized with NdeI and BamH to construct pPR-Vh(BGP)-H(Caf)-EpOR. The Suc-Vh-BGP(Caf)-EpOR DNA fragments were amplified using the primers Xba-Suc back and Sfl-EpOR HTM For and inserted into pBT-Vh(BGP)-EpOR linearized by XbaI and SfiI to obtain pBT-Vh(Caf-H(Caf))-EpOR.

The Suc-Vh(BGP)-EpOR DNA fragment was amplified using the primers Spe-Suc back and Nhel dERD2 For or Xba-Suc back and Nhel_dERD2 For to generate pPR-Vh(BGP)-EpOR. The Suc-Vh(BGP)-EpOR DNA fragment was amplified using the primers Spe-Suc back and Nhel_dERD2 For to generate pPR-Vh(BGP)-EpOR. The Suc-Vh(BGP)-EpOR DNA fragment was amplified using the primers Spe-Suc back and Nhel_dERD2 For to generate pPR-Vh(BGP)-EpOR. The Suc-Vh(BGP)-EpOR DNA fragment was amplified using the primers Spe-Suc back and Nhel_dERD2 For to generate pPR-Vh(BGP)-EpOR.
The amplified fragments were inserted into the pMD19 T-vector (Takara Bio Inc., Kusatsu, Japan), obtaining pMD19-5A7-VH1 and pMD19-5A7-VL1, respectively. The VH1(AFx)_1 and VL1(AFx)_1 DNA fragments were amplified using the primer sets VH 5A7_NcoI_Back/VH 5A7_Hind_For and VL 5A7_NcoI_Back/VL 5A7_Hind_For, with pMD19-5A7-VH1/pMD19-5A7-VL1 as the templates, respectively.

To construct the inverse pair, VH2(AFx)_2 and VL2(AFx)_2 fragments were amplified using Not1_VHback and Bam1_VHfor and Not1_VLback and Bam1_VLfor primer sets, respectively, with the same plasmids as templates. Both fragments were inserted into corresponding hybridoma cell lines, using the degenerate 5′-primers MHV-2 (for VH) and MKV-5 (Jones and Bendig, 1991) (for VL) combined with fixed 3′-primers MHC-uni (for VH) and mx-GSP (for VL), respectively. The DNA fragments were then subcloned into pBlueScript II (Toyobo) and the VH and VL nucleotide sequences were determined. The VH and VL DNA fragments were then separately amplified with #O157VH1-Rev and #O157VH1-For, and #O157VL1-Rev and #O157VL1-For primers, respectively, and spliced by OE-PCR into a desirable scFv gene fragment. This was re-amplified using the primers Bam-VLO157for and Not1-VH0157back using cloned scFv DNA as a template. The amplified scFv(O157) fragment was then inserted into the pPR-V(H(Caf))-EpoR plasmid linearized by Not1 and BamHI to construct pPR-ScFv(O157)-EpoR. We constructed pBT-ScFv(O157)-EpoR by amplifying scFv(O157) DNA using the primers Hind-ScFvO157for and Nco_ScFvO157back, then inserting it into pBT-V(H(Caf))-EpoR linearized with HindIII and NcoI.

To construct pLexA-CLY, the 8 × LexAOps LexA-binding sites and GAL1 promoter regions were amplified using LexA_SmaFor and LexA_Bam-back primers and pSH18-34 (Nova Lifetech Inc., Hong Kong, China) as a template. The amplified fragment was inserted into the multiple cloning site of the yeast Cluc reporter pCLY (ATTO, Tokyo, Japan) digested with BamHI and SmaI using In-Fusion HD cloning kits (Takara Bio Inc.).

Supplementary Table 1 shows the sequences of the synthesized primers (Eurofins Genomics K.K., Tokyo, Japan).

2.3. Preparation of Patrol Yeasts to detect small molecules

The constructed plasmids were transformed into S. cerevisiae NMY51 (Dualsystems) using the Frozen-EZ Yeast Transformation II Kit (Zymo Research Corp., Irvine, CA, USA). The transformed yeasts were grown on selective agar media (Minimal SD Base with dropout supplement, Trp- and Leu- for the β-galactosidase system; Trp-, Leu-, Ura-for the Cluc system; Takara Bio Inc.) to generate single colonies carrying the plasmids of interest. Yeast was grown in the liquid selection medium containing 100 μg/mL ampicillin overnight at 30 °C.

2.3.1. Beta-galactosidase reporter system

Overnight yeast cultures diluted to OD600 = 0.3 were grown in selection medium with or without various concentrations of antigens. Yeast cultures were then incubated for 16 h at 30 °C. Thereafter, yeast cultures (2.5 mL) were adjusted to OD600 = 1.0, then centrifuged at 800 × g for 2 min to obtain a precipitate. For the chemiluminescence assay, yeast precipitates were suspended in 500 μL of phosphate-buffered saline (PBS, pH 7.4) containing 2.5 mg/mL Zymolyase (100T; Nacalai Tesque, Kyoto, Japan), incubated for 1 h at room temperature, then disrupted for 10 s at 1 s intervals, 45% amplitude, 20 kHz on ice using a Q125 ultrasonicator (Qsonica LLC, Newtown, CT, USA).

2.3.2. Cluc reporter system

Yeast transformants were incubated in selection medium for ~48 h to reach OD600 = 5 (1 cm light path), then 25-μL portions were added to 3 mL of selection medium supplemented with 200 mM potassium phosphate buffer (pH 6.0). Yeast cultures were incubated with antigens at various concentrations for 25 h at 30 °C, then CLuc activity was measured.

2.4. Preparation of Patrol Yeasts to detect E. coli O157:H7

Shiga toxin-negative enterohemorrhagic E. coli O157:H7 (ATCC 43888) cultures were diluted to various concentrations based on OD600 measured with a V-730 spectrophotometer (Jasco, Tokyo, Japan), then colony-forming units (cfu)/mL were counted. A standard curve of the
linear relationship between OD$_{600}$ and the cfu/mL of O157:H7 was derived and used to estimate the numbers of cfu/mL based on the OD$_{600}$.

Yeasts harboring pBT- and pPR-ScFv(O157) plasmids were grown in a selection medium containing 100 μg/mL ampicillin at 30 °C overnight, centrifuged at 5000 x g for 5 min, resuspended in selection medium containing Zymolyase (0.25 mg/mL) and ampicillin (100 μg/mL) to OD$_{600}$ = 0.3, then incubated with gradient concentrations of E. coli O157:H7 for 16 h at 20 °C. A mixture of the cultured yeast and bacteria was centrifuged at 11,000 x g for 10 min, then E. coli cells in the supernatant were removed. The pelleted yeast cells were sonicated as described above. E. coli strains producing Shiga toxin were autoclaved at 121 °C for 15 min in PBS, then specificity was determined at 10$^2$ cfu/mL.

2.5. Chemiluminescence assay

Extracts of Patrol Yeast containing the β-galactosidase reporter system were obtained by sonication and mixed with AttoGlow β-galactosidase chemiluminescence substrate (Michigan Diagnostics, Royal Oak, MI, USA) at a 1:1 ratio (v/v) for 45–60 min at room temperature. Chemiluminescence was assayed in Costar 96-well half-area white plates (Corning Inc., Corning, NY, USA). The intensity of chemiluminescence triggered by 0.2 M NaOH was measured using an AB-2350 luminometer (ATTO, Tokyo, Japan) with an 0.2-s gate and calculated as the sum of 30 measurements taken over 30 min.

Cultured Patrol Yeast (20 μL) harboring the CLuc reporter was mixed with 80 μL of CLuc substrate in the buffer provided in the CL-S1000 CLuc substrate kit (ATTO). Luminescence intensity was measured immediately at room temperature using an AB-2350 luminometer with a 0.2-s gate. Luminescence intensity was calculated as the sum of 30 measurements taken over 15 min. The OD$_{600}$ of the yeasts for CLuc activity normalization was measured using an SH-1000 microplate reader (Corona Electric, Hitachinaka City, Japan) in transparent 96-well plates (200 μL/well) (Greiner Bio-One, Kremsmünster, Austria). Dose-response curves were fitted to the following four-parameter logistic equation: $y = d-((a-d)/(1+((c/x)^b))$, using ImageJ (Schneider et al., 2012) or GraphPad Prism7 (GraphPad Software Inc., San Diego, CA, USA). The limit of detection (LOD) was calculated as the concentration corresponding to the mean blank value plus 3 standard deviations (SDs) of the blank.

2.6. Flow cytometry

Yeast cells were collected by centrifugation at 700 x g for 2 min and suspended in 600 μL of PBS containing 2.5 mg/mL Zymolyase and 0.1 mg/mL 5-dodecanoylaminofluorescein di-β-d-galactopyranoside (C$_{12}$FDG) (Wako, Tokyo, Japan). After incubation for 1 h at room temperature and centrifugation at 700 x g for 2 min, the supernatant was discarded, then the cells were resuspended in PBS and measured using an SH-800 cell sorter (Sony, Tokyo, Japan). We measured 100,000 events per analysis. Products were excited at 488 nm and emission was detected using a 585/30 nm bandpass filter.

Patrol Yeast to detect E. coli O157 was incubated for 1 h with 2.5 mg/mL of Zymolyase to remove cell walls, then 10 nM streptavidin-phycoerythrin to label yeast cells with EpoR on the membrane, and 0.1 mg/mL C$_{12}$FDG to trace β-galactosidase activity. The cells were washed three times with PBS (as above) and assessed by flow cytometry (FCM). Phycoerythrin (PE) was excited at 488 nm and emission was detected using a 585/30 nm bandpass filter.

3. Results and discussion

3.1. Patrol Yeast detected bone Gla protein (BGP)

The model small antigen seaptide Bone Gla Protein (BGP)-C7 (derived from the C-terminal of human bone Gla protein or osteocalcin) was chosen as the target, and a Patrol Yeast was constructed to detect it. Transformed yeast cells were cultured with or without BGP-C7 antigen for 16 h at 30 °C, then β-galactosidase activity was measured. The V$_H$ domain in pBT (bait) and V$_I$ in pPR (prey) or V$_I$ in pBT and V$_I$ in pPR were constructed for the sensor proteins. Yeast cells harboring the plasmids were cultured and assayed using chemiluminescence. The results revealed a BGP-dependent increase in luminescence intensity in yeast cells harboring pBT-V$_H$(BGP)-EpoR and pPR-V$_I$(BGP)-EpoR (Fig. S2). Next, the Patrol Yeasts targeting food-related antigens were developed.

3.2. Patrol Yeasts detected caffeine

Construction of a Patrol Yeast to detect caffeine was attempted using the variable region of an anti-caffeine, single-domain antibody V$_H$(H(Caf)) to determine the feasibility of detecting small molecules such as caffeine and aflatoxins. Because the transmembrane (TM) signaling capability of sensor proteins is essential for detecting molecules outside cells, we used the EpoR D2 and TM regions to connect the V$_H$ and split ubiquitin inside the cells [pBT-V$_H$(H(Caf))-EpoR and pPR-V$_I$(H(Caf))-EpoR vectors]. Protein dimers that formed after exposing the V$_H$ receptor to caffeine united the tethered split ubiquitins. These became functional and cleaved the artificial transcription factor LexA-VP16, triggering transcription of the β-galactosidase reporter gene. Variants without the EpoR D2 domain retained only the TM domain linking V$_H$ and the split ubiquitin proteins (Fig. 2A). The activity of β-galactosidase in the yeast lysate was higher after an overnight incubation with, than without caffeine (Fig. 2B). Moreover, the Patrol Yeast without the D2 domain increased the signal-to-background ratio by lowering the background signal (Fig. S3).

Yeast cells expressing sensor proteins were incubated overnight with various concentrations of caffeine, then disrupted by sonication to measure β-galactosidase activity. Higher caffeine concentrations resulted in more β-galactosidase activity (Fig. 2B), indicating that caffeine binding to the sensor proteins enhanced the β-galactosidase expression. The estimated EC$_{50}$ was 1.4 μM, and the limit of detection (LOD) was 27 nM after fitting into a four-parameter logistic equation. The Patrol Yeast also detected the concentrations of caffeine in several Coca-Cola® beverages or caffeine-free Coca-Cola® spiked with caffeine (Fig. S4).

We analyzed β-galactosidase activity using the membrane-permeable substrate C$_{12}$FDG and FCM to determine reporter activation at the cellular level. Cells with increased fluorescence intensity detected by FCM indicated antigen-induced β-galactosidase activity (Fig. S5). The median fluorescence intensity of the yeast population increased 5-fold after incubation with higher caffeine concentrations.

3.3. Patrol yeasts detected aflatoxins

The V$_H$(H(Caf)) variable regions were replaced with V$_H$(AFx) of the anti-aflatoxin antibody in pBT-V$_H$(AFx)-EpoR and pPR-V$_I$(AFx)-EpoR to develop a Patrol yeast that detects aflatoxins. Thus, sensor proteins detect aflatoxins in a similar split-ubiquitin mechanism with the EpoR domain and the TM linker described above (Fig. 2A). The dose-dependence of Patrol Yeast harboring these plasmids was determined as chemiluminescence emission. Aflatoxins B1 and M1 activated the split-ubiquitin mechanism in the Patrol Yeast cells harboring pBT-V$_H$(AFx)-EpoR + pPR-V$_I$(AFx)-EpoR, which dose-dependently detected β-galactosidase activity (Fig. 3B and C). The estimated LODs for aflatoxins B1 (AFB1) and M1 (AFM1) were 0.003 and 0.033 nM after curve fitting, respectively, which were sufficiently low considering permissible
afattoxin contents in foods under the AFB1 worldwide and AFM1 European Union regulations of 3–64 and 0.15 nM, respectively (Clarke et al., 2014; Henry et al., 2002).

### 3.4. Patrol Yeast detected E. coli O157

Next, a system corresponding to that of enterohemorrhagic E. coli O157 was constructed to determine the ability of Patrol Yeast to detect...
larger targets. Because the target was not a small molecule, we used the scFv of the anti-O157 antibody as the detector by replacing the V<sub>H</sub>(H(Caf)) domain (Fig. 4A). Because one E. coli cell carries multiple copies of a specific sugar epitope on its outer membrane, scFvs should be combined to activate the ubiquitin mechanism and provide O157-dependent β-galactosidase activity. However, the yeast cell wall should be at least partially destroyed to allow O157 to reach the yeast cell membrane and emit the TM signal. First, the yeast was grown with various concentrations of live Shiga toxin-negative E. coli O157:H7. We finally detected O157:H7 dose-dependent β-galactosidase activity as chemiluminescence emission after optimizing Zymolyase digestion (Fig. 4B and S6a). Prolonged co-culture of the yeast and E. coli O157:H7 for 16 h at 20 °C with 0.25 mg/mL of Zymolyase resulted in intense signals. As expected, Zymolyase was essential to obtain O157-dependent signals (Fig. S6b).

The estimated LOD of O157:H7 detection was 2.5 × 10<sup>6</sup> CFU/mL. The specificity of Patrol Yeast for O157 was investigated using inactivated and autoclaved Shiga toxin-producing enterohemorrhagic E. coli O26, O111, and O157 isolates. Fig. S7 shows that the signals generated from live and heat-inactivated O157 were essentially the same after incubating Patrol Yeast in medium containing antibiotic ampicillin. The results showed that only the O157 strains generated a significant increase in luminescence signals, indicating that only specific binding between O157 and the Patrol Yeast generated β-galactosidase activity (Fig. S8).

We also assessed the ability of Patrol Yeast digested by Zymolyase to detect O157 as fluorescence emission. We eliminated interference from O157:H7 cells and visualized yeast cells harboring sensor proteins by labeling the EpoR domain of the sensor proteins on cell membranes with streptavidin/phycoerythrin (PE) and a biotin-conjugated anti-EpoR antibody. The FC results showed increased red fluorescence from PE in all yeast groups harboring plasmids for sensor proteins, indicating sensor protein expression and integration into the membrane. The intensity of green fluorescence generated by β-galactosidase increased with higher concentrations of O157:H7 (Fig. S9). The increased ratio of the fluorescence intensity (green/red) corresponded to higher O157:H7 concentrations, indicating interaction between O157:H7 and sensor proteins on the cell membrane of the Patrol Yeast that activated β-galactosidase expression.

3.5. Patrol Yeasts with a secreted luciferase reporter system

We used a secreted Cypridina noctiluca luciferase (CLuc) reporter system (Yamagishi et al., 2006) to further widen the applications of the Patrol Yeast. The CLuc reporter has been included in yeast for several types of assays (Kanjou et al., 2007; Yamagishi et al., 2006). The plasmids, pBT-<i>V</i><sub>H</sub>(H(Caf))-dERD2, pPR-<i>V</i><sub>H</sub>(H(Caf))-dERD2, and pLexA-CLY were co-transformed into the yeast strain NY51 to obtain a Patrol Yeast with secreted CLuc to detect caffeine. The LexA-VP16 artificial transcription factor liberated after caffeine recognition was predicted to trigger CLuc expression from the pLexA-CLY plasmid. The activity of CLuc can be directly detected in cultured yeast cells without a separation procedure. Fig. 5 shows that dose-dependent CLuc signals were found in the yeast cultures. Notably, CLuc activity was detected in yeast cultures containing 10 nM caffeine, which was not found in the β-galactosidase reporter system. After fitting into a four-parameter logistic equation, the estimated LOD of this system was 1.5 nM, which was one order of magnitude more sensitive than the β-galactosidase reporter with Atto-Glow substrate.

4. Conclusions

A novel yeast-based system was developed to detect toxins and microorganisms in food and beverages. Compared with other means of analyzing food contamination such as GC/MS/MS, yeast renders our new system inexpensive and more convenient as it is manageable and the technology for large-scale yeast production is mature. This is also an advantage over mammalian cell-based sensors that require expensive media and careful culture in a humidified atmosphere. Furthermore, the signal-on biosensor created for Patrol yeast led to rapid detection with good sensitivity. An organism-based sensor also reduces health and environmental risks during the detection process. Patrol yeast could detect multiple targets enabled by minor modifications to the sensor proteins. Caffeine detection was sufficiently sensitive for analyses of most common beverages, and sensitivity for aflatoxin detection also met mainstream food contamination regulations worldwide (Clarke et al., 2014; Henry et al., 2002), indicating the feasibility of Patrol Yeast in terms of food safety. The versatility of the Patrol yeast system was also shown by its ability to detect large targets such as E. coli O157. In addition to the established β-gal reporter, the secreted luciferase reporter (CLuc) further improved the feasibility of the Patrol Yeast system as it reduced the risk of generating aerosols and equipment requirements during practical operations.

One limitation of the Patrol yeast platform is that incubations are relatively longer than that in conventional analytical methods. However, incubations can proceed overnight which avoids hands-on time for sample analysis, and they take less time overall than mammalian systems. In conclusion, we believe that the Patrol Yeast system will contribute to the field of food safety as a sensitive, cost-effective, and manageable tool with which to detect and monitor a wide spectrum of targets.

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CRediT authorship contribution statement


Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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