2011

Green fluorescent protein-based biosensor to detect and quantify stress responses induced by DNA-degrading colicins

Sam Abraham
University of Wollongong, sabraham@uow.edu.au

James Chin
Elizabeth Macarthur Agricultural Institute

Huub J. M. Brouwers
Elizabeth Macarthur Agricultural Institute

Bernadette Turner
Elizabeth Macarthur Agricultural Institute

Ren Zhang
University of Wollongong, rzhang@uow.edu.au

See next page for additional authors

Green fluorescent protein-based biosensor to detect and quantify stress responses induced by DNA-degrading colicins

Abstract

Here we report the development of a whole-cell biosensor to detect and quantify the induction of the SOS response activated by DNA-degrading colicins. This biosensor utilizes the SOS-responsive cda promoter to regulate the expression of green fluorescent protein. The biosensor assay revealed induction of stress for all DNA-degrading reference colicins (E2, E7, and E8).

Publication Details

Abraham, S., Chin, J., Brouwers, H. J.M.., Turner, B., Zhang, R. & Chapman, T. A.. (2011). Green fluorescent protein-based biosensor to detect and quantify stress responses induced by DNA-degrading colicins. Applied and Environmental Microbiology, 77 (18), 6691-6693.

Authors

Sam Abraham, James Chin, Huub J. M. Brouwers, Bernadette Turner, Ren Zhang, and Toni A. Chapman

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 2011, p. 6691–6693 Vol. 77, No. 18
0099-2240/11/$12.00 doi:10.1128/AEM.00534-11

Green Fluorescent Protein-Based Biosensor To Detect and Quantify Stress Responses Induced by DNA-Degrading Colicins▽†

Sam Abraham,[1,2] James Chin,[1]‡ Huub J. M. Brouwers,[1] Bernadette Turner,[1] Ren Zhang,[2] and Toni A. Chapman[1]*

Microbiological Diseases and Diagnostics Research, New South Wales Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, New South Wales 2568,[1] and School of Biological Sciences, University of Wollongong, Wollongong, New South Wales 2500,[2] Australia

Received 9 March 2011/Accepted 18 July 2011

Here we report the development of a whole-cell biosensor to detect and quantify the induction of the SOS response activated by DNA-degrading colicins. This biosensor utilizes the SOS-responsive *cda* promoter to regulate the expression of green fluorescent protein. The biosensor assay revealed induction of stress for all DNA-degrading reference colicins (E2, E7, and E8).

One of the mechanisms manifested by *Escherichia coli* in combat is the production of bacteriocins such as colicins and microcins (6). The highly specific and potent bacteriocins are produced in response to stressful conditions and aimed against other clones which do not have defensive weaponry or immunity genes. The highly diverse and well-studied colicins are proposed to play a significant role in maintaining and mediating the population dynamics within the *E. coli* community (13, 15).

The colicins kill the target bacteria via a range of mechanisms, including the formation of pores in the cytoplasmic membrane (colicins A, B, E1, Ia, Ib, K, N, and U), nonspecific degradation of DNA (colicins E2, E7, E8, and E9), degradation of RNA (colicins E3, E4, E5, E6, and D), and inhibition of peptidoglycan synthesis (col M) (3). In this study, we establish the use of a *cda-gfp* whole-cell biosensor to detect and quantify the stress induced by DNA-degrading colicins that kill the target by activating a genotoxic stress response.

Strains used in the study were as follows: MG1655/pANO1::*cda′* [*Escherichia coli* K-12 MG1655 carrying pANO1::*cda′*, containing a transcriptional fusion between the *cda* promoter and *gfp*mut3 (Amp^r Cm^r)] (9) as well as BZB 2101 (reference colicin A), BZB 2102 (reference colicin B), BZB 2103 (reference colicin D), BZB 2104 (reference colicin E1), BZB 2125 (reference colicin E2), BZB 2106 (reference colicin E3), BZB 2107 (reference colicin E4), BZB 2108 (reference colicin E5), BZB 2150 (reference colicin E6), Pap 247 (reference colicin E8), BZB 2114 (reference colicin Ia), BZB 2115 (reference colicin Ib), BZB 2116 (reference colicin K), Pap 1 (reference colicin M), and BZB 1011 (colicin-sensitive strain) (all from David Gordon) (see Table S1 in the supplemental material).

* Corresponding author. Mailing address: P.M.B. 4008, Narellan 2567, NSW, Australia. Phone: 02 46406219. Fax: 02 46406384. E-mail: toni.chapman@industry.nsw.gov.au.

‡ Present address: Plant Breeding Institute, Faculty of Agriculture, Food & Natural Resources, University of Sydney, Cobbity, NSW 2570, Australia.

† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 29 July 2011.

For the production of colicin supernatants, we utilized the method of Gordon et al. (7) with the additional modification of filter sterilizing the supernatant before storing it at −80°C for 48 h.

The preparation of the biosensor for the stress response assay was performed as described by Norman et al. (9). All stress assays were performed in triplicate in 96-well microtiter plates using 100 μl of ~0.2-optical-density (OD) biosensor with 50 μl of different concentrations of colicin supernatant diluted in LB. The microtiter plate was then incubated at 37°C (Wallac Victor^2 1420; PerkinElmer, Australia) with periodic shaking, and the fluorescence intensity and the OD at 600 nm of the samples were measured every 15 min for 3 to 4 h. The fluorescence intensity was measured at an excitation of 485 nm and an emission of 535 nm. Endpoint enumeration of the biosensor was performed to validate the survival of biosensor after each assay. Induction factor (IF) was calculated by dividing the mean fluorescent intensity of the samples with stressors by the mean fluorescent intensity of the negative-control sample (LB plus biosensor) at the same time point. An induction was considered significant when an increase of induction factor by 0.5 was observed (IF $\geq$ 1.5), coupled with an observation of dose response similar to the International Organization for Standardization (ISO) standardized SOS chromotest (10, 12). Relative optical density (OD_x/OD_o) was calculated by dividing the mean optical density of the sample with the stressors (OD_x) by the optical density of the negative-control sample (OD_o) (LB plus biosensor) at the same time point. All values were means ± standard deviations for $n = 3$.

To evaluate the ability of colicins to induce a stress response in a genotoxin-responsive biosensor, we characterized the responses of representative reference colicins that kill the target by different mechanisms. The colicin-mediated induction of a stress response in the biosensor was assessed by coincidentally measuring fluorescence intensity over time in both colicin-treated and control cells (LB broth).

The inductions of stress in the biosensor by various concentrations of the DNA-degrading colicins E2, E7, and E8 were compared by measuring the induction factor (IF) and the relative optical density over time (Fig. 1). The ability of these

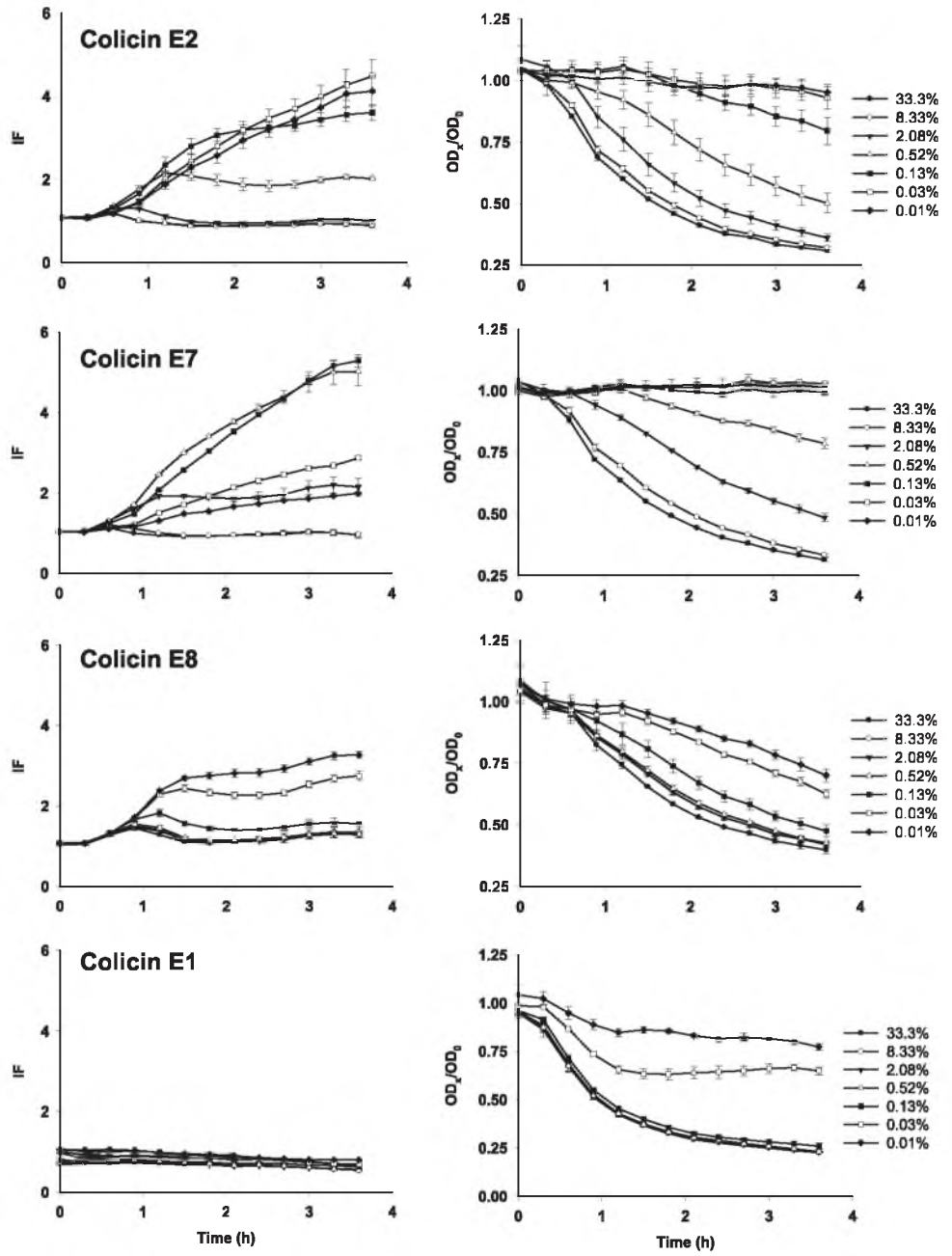

FIG. 1. Induction factor (IF) (left) and relative optical densities (OD_x/OD_o) (right) of dose-response assay of biosensor MG1655/pANO1::*cda'* when treated with various concentrations of DNA-degrading colicins E2, E7, and E8 and pore-forming colicin E1. The percent values in the key represent the percentage of colicin supernatant present in each sample. The representative colicins for each plot are indicated above the respective panel. All values were means ± standard deviations for $n = 3$.

colicins to induce a stress response in the biosensor was apparent only 1 h after the addition of colicin supernatant. The dose-response curve of colicins E2, E7, and E8 revealed significant induction of genotoxic stress on the target biosensor denoted by an IF above 3.

An IF above 4 was obtained both for E2 colicin supernatant at concentrations of 0.03% and 0.01% and for E7 colicin supernatant at concentrations of 0.52% and 0.13%. For colicin E8 culture supernatant, the maximum IF of 3.5 was observed at a concentration of 0.01%. At concentrations above 2.08% of colicin supernatants, an initial increase in the IF was observed followed by a continuous decline after a period of time. A continuous decrease in the relative optical density was also observed above 2.08% of colicin supernatants. A steady increase in IF over time was observed until 3 h from concentrations of 0.13% to 0.01% for colicin E2, 0.52% to 0.13% for colicin E7, and 0.03% to 0.01% for colicin E8; however, no significant increase was noticed after 3 h.

The pore-forming colicins (A, B, E1, Ia, and Ib) and RNA-degrading colicins (E3, E4, E5, E6, and D) were also tested for

their ability to induce the genotoxic biosensor, but none did. A representative plot of data for the pore-forming colicin E1 is shown in Fig. 1. The relative optical densities and the endpoint enumeration demonstrated effective inhibition of growth and killing of the biosensor by the pore-forming and RNA-degrading colicin supernatants (data not shown). Supernatant from the colicin-sensitive strain BZB1011 was used as a negative control, and this did not induce a genotoxic stress response in the biosensor (see Fig. S1 in the supplemental material).

Bacterial community interactions are complex, with bacteria working together and against each other to maintain themselves within the environment in which they live (1, 8, 14). This work focused on the use of a whole-cell biosensor, MG1655/pANO1::*cda'*, for real-time profiling of the kinetic and dynamic measurement of colicin-induced stress responses.

The biosensor MG1655/pANO1::*cda'* can report in real time the cytotoxicity induced by colicins and the activation of stress response mechanisms in the target. The induction of stress responses using reference colicins on the colicin-sensitive biosensor demonstrated that only DNA-degrading colicins induce the SOS response on this biosensor. The time- and dose-dependent responses of the biosensor to treatment with DNA-degrading reference colicin extracts (colicins E2, E7, and E8) were initially similar; however, the final induction factors were unique to each colicin, which may relate to differences in colicin concentrations in each extract.

The optimum assay time for measurement of colicin-mediated stress response was between 90 and 120 min. The biosensor assay offers fast, real-time, and specific detection of colicin action. The biosensor assay allows the measurement of real-time responses of colicin action on the basis of the stress response and cytotoxicity detected by continuously monitoring changes in fluorescence and optical density, respectively.

This biosensor clearly offers advantages over traditional methods and yet is not applicable to all colicins (7, 11). However, if an array of biosensors is constructed with other stress-responsive promoters such as *rpoS*, *grpE*, *cspA*, *ibp*, and *uspA*, which respond to general stress, heat shock, cold shock, cytoplasmic stress, and starvation, respectively (2, 4, 5), the biosensor system could be effectively used to detect and quantify stress induced by any colicins.

Sam Abraham is a recipient of a Ph.D. scholarship from International Animal Health Products, Pty. Ltd., Australia.

We thank Lars Hestbjerg Hansen, University of Copenhagen, for making the MG1655 pANO1::*cda'* biosensor available. We also thank David Gordon, Australian National University, for providing the colicin reference and colicin-sensitive strains. Special thanks go to Mark R. Wilson, School of Biological Sciences, University of Wollongong, for critical reading of the manuscript.

REFERENCES

1. **Backhed, F., R. E. Ley, J. L. Sonnenburg, D. A. Peterson, and J. I. Gordon.** 2005. Host-bacterial mutualism in the human intestine. Science **307:**1915–1920.
2. **Bianchi, A. A., and F. Baneyx.** 1999. Stress responses as a tool to detect and characterize the mode of action of antibacterial agents. Appl. Environ. Microbiol. **65:**5023–5027.
3. **Cascales, E., et al.** 2007. Colicin biology. Microbiol. Mol. Biol. Rev. **71:**158–229.
4. **Funabashi, H., et al.** 2002. Non-destructive monitoring of rpoS promoter activity as stress marker for evaluating cellular physiological status. J. Biotechnol. **95:**85–93.
5. **Gawande, P. V., and M. W. Griffiths.** 2005. Effects of environmental stresses on the activities of the uspA, grpE and rpoS promoters of *Escherichia coli* O157:H7. Int. J. Food Microbiol. **99:**91–98.
6. **Gordon, D. M., E. Oliver, and J. Littlefield-Wyer.** 2006. The diversity of bacteriocins in gram-negative bacteria, p. 5–18. *In* M. Riley and M. Chavan (ed.), Bacteriocins: ecology and evolution. Springer, Berlin, Germany.
7. **Gordon, D. M., M. A. Riley, and T. Pinou.** 1998. Temporal changes in the frequency of colicinogeny in *Escherichia coli* from house mice. Microbiology **144:**2233–2240.
8. **Guarner, F., and J. Malagelada.** 2003. Gut flora in health and disease. Lancet **361:**512–519.
9. **Norman, A., L. H. Hansen, and S. J. Sørensen.** 2005. Construction of a ColD cda promoter-based SOS-green fluorescent protein whole-cell biosensor with higher sensitivity toward genotoxic compounds than constructs based on recA, umuDC, or sulA promoters. Appl. Environ. Microbiol. **71:**2338–2346.
10. **Ostergaard, T. G., L. H. Hansen, M.-L. Binderup, A. Norman, and S. J. Sorensen.** 2007. The cda GenoTox assay: a new and sensitive method for detection of environmental genotoxins, including nitroarenes and aromatic amines. Mutat. Res. **631:**77–84.
11. **Pugsley, A. P.** 1985. *Escherichia coli* K12 strains for use in the identification and characterization of colicins. J. Gen. Microbiol. **131:**369–376.
12. **Quillardet, P., and M. Hofnung.** 1993. The SOS chromotest: a review. Mutat. Res. **297:**235–279.
13. **Riley, M. A., and D. M. Gordon.** 1999. The ecological role of bacteriocins in bacterial competition. Trends Microbiol. **7:**129–133.
14. **Tenaillon, O., D. Skurnik, B. Picard, and E. Denamur.** 2010. The population genetics of commensal *Escherichia coli*. Nat. Rev. Microbiol. **8:**207–217.
15. **Walker, D., et al.** 2004. Transcriptional profiling of colicin-induced cell death of *Escherichia coli* MG1655 identifies potential mechanisms by which bacteriocins promote bacterial diversity. J. Bacteriol. **186:**866–869.