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Use of *Saccharomyces cerevisiae* BLYES Expressing Bacterial Bioluminescence for Rapid, Sensitive Detection of Estrogenic Compounds

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An estrogen-inducible bacterial *lux*-based bioluminescent reporter was developed in *Saccharomyces cerevisiae* for applications in chemical sensing and environmental assessment of estrogen disruptor activity. The strain, designated *S. cerevisiae* BLYES, was constructed by inserting tandem estrogen response elements between divergent yeast promoters GPD and ADH1 on pUTK401 (formerly pUA12B7) that constitutively express *luxA* and *luxB* to create pUTK407. Cotransformation of this plasmid with a second plasmid (pUTK404) containing the genes required for aldehyde synthesis (*luxCDE*) and FMN reduction (*frp*) yielded a bioluminescent bioreporter responsive to estrogen-disrupting compounds. For validation purposes, results with strain BLYES were compared to the colorimetric-based estrogenic assay that uses the yeast *lac*Z reporter strain (YES). Strains BLYES and YES were exposed to 17β-estradiol over the concentration range of 1.2×10^{-8} through 5.6×10^{-12} M. Calculated 50% effective concentration values from the colorimetric and bioluminescence assays ($n = 7$) were similar at $(4.4 \pm 1.1) \times 10^{-10}$ and $(2.4 \pm 1.0) \times 10^{-10}$ M, respectively. The lower and upper limits of detection for each assay were also similar and were approximately 4.5×10^{-11} to 2.8×10^{-9} M. Bioluminescence was observed in as little as 1 h and reached its maximum in 6 h. In comparison, the YES assay required a minimum of 3 days for results. Strain BLYES fills the niche for rapid, high-throughput screening of estrogenic compounds and has the ability to be used for remote, near-real-time monitoring of estrogen-disrupting chemicals in the environment.

Evidence suggests a wide variety of xenobiotic compounds (e.g., pesticides, plasticizers, and synthetic hormones) and naturally occurring chemicals possess steroid-like activities that lead to the disruption of the endocrine system in vertebrates (5, 8, 9, 15, 17, 34). In response to public health concerns, the United States Congress directed the Environmental Protection Agency (EPA) to develop a screening program for evaluating the potential of pesticides and other substances to induce hormone-related health effects (Food Quality Protection Act [Public Law 104-170]). This screening approach is enormous in scope, with the EPA estimating that 87,000 existing and new chemicals require testing (7). Furthermore, the U.S. Geological Survey recently reported a low-level occurrence of steroid growth hormones in 80% of 139 water systems examined in the United States (16). Other developed countries, including the United Kingdom, Germany, The Netherlands, Italy, Canada, Brazil, and Japan, share the problem of endocrine-disrupting chemicals in the environment.

Several in vivo mammalian assays (reviewed in reference 23) and in vitro assays (reviewed in references 8 and 36) exist for measuring estrogenic effects. In vitro assays fall into the following broad categories: competitive ligand binding assays, cell proliferation assays, postconfluent cell accumulation, induction of protein expression/enzyme activities, and recombinant receptor/reporter gene assays (for a complete review, see reference 36). Recombinant receptor/reporter gene assays are designed to detect the induction or repression of a biological process via specific endocrine receptors. These assays usually have a high responsiveness and sensitivity and can be used to assess the relative potency of alleged receptor-mediated agonists and antagonists (4, 36).

A widely used receptor/reporter assay for detecting estrogenic compounds is the yeast estrogen screen (YES) (26). The *Saccharomyces cerevisiae* strain contains the human estrogen receptor (hER-α) and a plasmid-based estrogen response element (ERE)-*lacZ* reporter fusion. When an estrogen-like compound binds to the estrogen receptor protein, it in turn binds to the ERE, inducing transcription of *lac*Z. β-Galactosidase transforms the chromogenic substrate chlorophenol red-β-D-galactopyranoside (CPRG) to a red product measured by absorbance at 540 nm. This assay has been used extensively to

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TABLE 1. *Escherichia coli* and *Saccharomyces cerevisiae* strains and plasmids used in this study

Strain or plasmid	Description[a]	Source or reference
E. coli strains		
DH5α	ϕ80d*lacZΔM15 recA1 endA1 gyrA96 thi-1 hsdR17* (r_K^- m_K^-) *supE44 relA1 deoR* Δ(*lacZYA-argF*)*U169*	Promega
Transformax	F^- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80*dlacZΔM15*	Epicentre, Madison, Wis.
EC100	Δ*lacX74 recA1 endA1 araD139* Δ(*ara, leu*)*7697 galU galK* λ^- *rpsL nupG*	
S. cerevisiae strains		
YES (*hER-lacZ*)	*Mat***a** *leu2 his3*; ERE-*lacZ* reporter plasmid and human estrogen receptor gene in the chromosome	26
hER	Strain YES without the ERE-*lacZ* reporter plasmid	26
BLYES	*S. cerevisiae* hER containing pUTK404 and pUTK407	This study
Plasmids		
pCR2.1 TOPO	Ap^r Kn^r, TA cloning vector	Invitrogen
pUTK401	pUA12B7 (pBEVY-U containing *luxA* and *luxB*)	12
pUTK404	pLCIRESDEIRESfrp (pBEVY-L harboring *luxCDEfrp*)	12
pUTK407	pUTK401 containing 2 ERE elements between the GPD and ADH1 constitutive promoters	This study
pUTK408	pCR2.1 TOPO containing GPDEREADH	This study

[a] Abbreviations: Ap^r, ampicillin resistant; Kn^r, kanamycin resistant.

measure estrogenic responses to polychlorinated biphenyls and hydroxylated derivatives (18, 28, 30), polynuclear aromatic hydrocarbons (31), and other compounds (32), as well as detection of estrogens in wastewater treatment systems (18) and dairy manure (25). In addition, the YES assay has been used for screening contaminated water for antiestrogenic activity (24). Although proven effective for the in vitro determination of estrogenic activity, the standard colorimetric YES assay's incubation time of 3 to 5 days (29) is impractical when considering the 87,000 chemicals requiring tier I screening. To overcome this issue, a bioluminescent version of this reporter has been constructed.

Bioluminescent bioreporter technology based on activation of gene fusions using the firefly (*luc*) or bacterial (*lux*) luciferase is as well established as *lacZ*, *cat*, or *gfp* reporter systems (6). The *luc* and *lux* reporters offer unique capabilities for functional transcriptional profiling (22, 35), in vivo monitoring of transcriptional logic gates (33), whole-body imaging (10, 11, 13), and reagentless microluminometer-based hybrid bio/silico sensors (3). Reporter constructs based on the complete bacterial *lux* cassette (*luxCDABE*) offer the distinct advantage over the *luc* genetic system of autonomous light generation without the requirement for exogenous substrate addition or secondary excitation. Further, the bacterial bioluminescence reaction generates a visible light signal that can be detected easily and quantified within hours rather than days, making it more amenable to rapid, high-throughput screening protocols.

Recently, Gupta and coworkers functionally expressed the *luxA*, *-B*, *-C*, *-D*, and *-E* genes from *Photorhabdus luminescens* and the *frp* gene from *Vibrio harveyi* in *Saccharomyces cerevisiae* (12). This bioreporter was engineered using two pBEVY yeast expression vectors (20), which allowed bidirectional constitutive expression of the individual *luxA*, *-B*, *-C*, *-D*, and *-E* genes. The *luxA* and *luxB* genes were independently expressed from divergent yeast constitutive promoters GPD and ADH1 on pBEVY-U. The *luxCD* and *luxE-frp* genes were independently expressed from a second plasmid (pBEVY-L), also using the GPD and ADH1 promoters. An internal ribosome entry site (IRES) was inserted between the *luxC* and *luxD* genes and the *luxE* and *frp* genes. The IRES allows translation of multiple genes from a single promoter in eukaryotes (14). This present work extends the bioreporter of Gupta and coworkers (12) by developing an estrogen-responsive yeast-based bioluminescent bioreporter and demonstrating its usefulness against known estrogenic and nonestrogenic compounds.

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. Strains and plasmids used in this study are listed in Table 1. *Escherichia coli* DH5α, used as a host for plasmid construction and maintenance, was grown in Luria-Bertani (LB) broth at 37°C with or without 100 μg ampicillin/ml, depending on the requirement for plasmid maintenance.

YPD liquid medium (1% yeast extract, 2% peptone, 2% glucose) was used for routine growth of plasmid-free *S. cerevisiae* strains. *S. cerevisiae* strains harboring plasmids with leucine and uracil selective markers were grown in modified minimal medium without leucine and uracil (YMM leu^-, ura^-) (26).

Chemicals. 17β-Estradiol (98% purity), 17α-estradiol, 17α-ethynyl estradiol (98% purity), diethylstilbestrol (DES; 99% purity), estrone (99% purity), 4,4′-cyclohexlidene bisphenol (98% purity), 4-andostrenedione (98% purity), and ethanol were purchased from Sigma-Aldrich Chemical Company (St. Louis,

TABLE 2. Oligonucleotide primer sequences used for the construction of plasmid pUTK407

Primer designation	Primer sequence[a]
ADHR	5′-CCCGGGCCCGGGGAGTTGATTGTATGCTTGGTATAGCTTGAAATATTGTGC-3′
EREADHF	5′-*CAGGTCACTGTGACCTCTCGAGGTCACTGTGACCT*GCCGGCATTCTTTTCTTTTTTTTTCTTTTCTCTCTCCCCCG-3′
EREGPDF	5′-GCCGGC*CAGGTCACTGTGACCTCTCGAGGTCACTGTGACCT*CCGGGAGTTTATC-3′
GPDR	5′-GGATCCGTCGAAACTAAGTTCTTGGTGTTTTAAAAC-3′

[a] Newly generated restriction sites are underlined. ERE sequences are italicized.

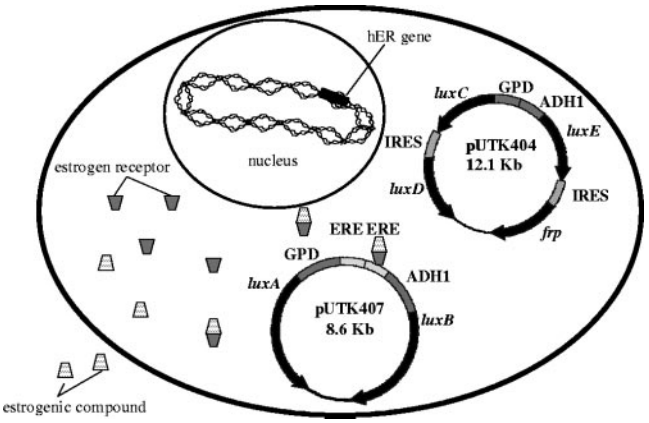

FIG. 1. Schematic representation of *S. cerevisiae* BLYES. Estrogenic compounds cross the cell membrane and bind to the estrogen receptor. This complex interacts with the ERE, initiating transcription of *luxA* and *luxB*. The gene for the human estrogen receptor is located on the chromosome.

MO). *para*-Nonylphenol was purchased from Supelco (Bellefonte, PA). Stock solutions of each chemical were made in ethanol.

Molecular biology techniques. DNA manipulations were performed according to standard protocols (27). Plasmids were transformed into *E. coli* and *S. cerevisiae* by electroporation using ECM600 (BTX Inc., Holliston, MA) as described by the manufacturer. For *E. coli* strains, electroporation conditions were as follows: charging voltage of 2.5 kV, resistance of 125 Ω, capacitance of 50 μF, pulse length of 5 ms. Immediately following transformation, 960 μl of SOC or LB medium was added and cells were allowed to recover at 37°C with shaking at 200 rpm for at least 1 hour. For *S. cerevisiae*, cells were prepared according to the manufacturer's instructions (BTX protocols). Cells were transformed with 300 to 500 ng of each plasmid DNA. Electroporation conditions were the same as for *E. coli*. Immediately following transformation, 1 ml cold 1 M sorbitol was added to the transformed cells. After 10 min, cells were plated on YMM (leu^-, ura^-) noble agar plates.

Plasmid isolation was performed using Wizard mini- or midi-prep kits (Promega, San Luis Obispo, CA) or the RPM yeast plasmid isolation kit (Bio101 Inc., Carlsbad, CA). PCR was performed in 25-μl volumes using Ready-to-Go PCR beads (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) and the oligonucleotide primers listed in Table 2. DNA sequencing was performed with an ABI Big Dye Terminator cycle sequencing reaction kit on an ABI 3100 DNA sequencer (Perkin-Elmer, Inc., Foster City, CA).

Construction of strain BLYES. *S. cerevisiae* strain BLYES contains the plasmids pUTK404 and pUTK407 and the hER-α gene in the chromosome (Fig. 1). The construction of pUTK404 has been described previously (12). Briefly, this plasmid contains the *luxC*, *-D*, and *-E* genes from *Photorhabdus luminescens* and the flavin oxidoreductase gene (*frp*) from *Vibrio harveyi* for provision of the $FMNH_2$ cofactor required for the bioluminescent reaction. The translation of *luxD* and *frp* genes is mediated by inclusion of a yeast IRES (14).

Sequential PCRs were used to insert tandem ERE between the bidirectional constitutive promoters of pUTK401 (formerly pUA12B7 [12]). In the first PCR, promoter GPD was amplified using primers GPDR and EREGPDF (Table 2; Fig. 2). Primer EREGPDF contains the entire sequence of the tandem ERE. Primer GPDR contains a unique BamHI site at the 5′ end. This 25-μl PCR mixture included Ready-to-Go PCR beads (Amersham Biosciences Corp., Piscataway, NJ) with 250 nM EREGPDF primer, 250 nM GPDR primer, and 1 ng pUTK401 template. A stringent touchdown thermal cycler program was used to amplify this promoter: 95°C for 15 min of initial denaturation, followed by 15 cycles of denaturation at 95°C for 30 s, annealing at 72°C for 30 s, and elongation at 72°C for 30 s. The annealing temperature was reduced by 1°C in each of the first 15 cycles. Then followed 30 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 30 s, and elongation at 72°C for 30 s. There was a final elongation step at 72°C for 2 min.

In the second PCR, the promoter ADH1 was amplified using primers ADHR and EREADHF (Table 2; Fig. 2). Primer EREADHF contains the entire sequence of the tandem ERE. The 25-μl PCR mixture consisted of 1× buffer D (FailSafe PCR system; Epicentre, Madison, WI), 250 nM primer EREADHF, 250 nM primer ADHR, 1 ng pUTK401 template, and 2.5 U of FailSafe PCR enzyme mix. A stringent touchdown thermal cycler program was used: 95°C for 15 min of initial denaturation, followed by eight cycles of denaturation at 95°C for 2 min, annealing at 72°C for 30 s, and elongation at 72°C for 1 min. The annealing temperature was reduced by 1°C in each of the first eight cycles. Then

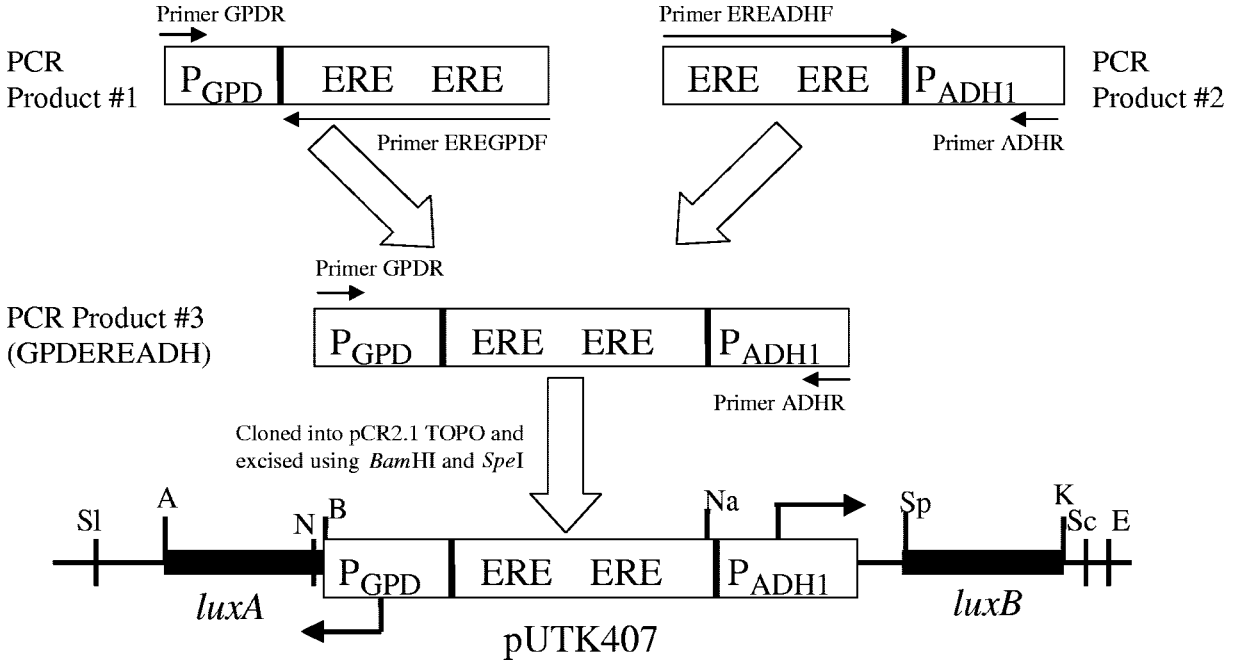

FIG. 2. PCR and cloning strategy for generating pUTK407. Sequential PCR steps were used to generate GPDEREADH. This construct was cloned into the BamHI-SpeI site of pUTK401 to generate pUTK407. Restriction site abbreviations: A, AvrII; B, BamHI; E, EcoRI; K, KpnI; N, NotI; P, PstI; Sc, SacI; Sl, SalI; Sp, SpeI; X, XmaI. Figure is not drawn to scale.

followed 25 cycles of denaturation at 95°C for 1 min followed by annealing at 65°C for 30 s and elongation at 72°C for 1 min. There was a final elongation step at 72°C for 4 min.

Each PCR product was gel purified (QIAquick gel extraction kit; QIAGEN Inc., Valencia, CA), quantified, and combined in a third PCR to amplify the entire GPD-ERE-ADH region (Fig. 2). This 25-μl PCR mixture consisted of 1× buffer D, 250 nM primer GPDR, 250 nM primer ADHR, 18 ng purified EREGPD from the first PCR, 20 ng purified EREADH from the second PCR, and 2.5 U of FailSafe PCR enzyme mix. The touchdown program used for this reaction was 95°C for 15 min of initial denaturation, followed by eight cycles of denaturation at 95°C for 2 min, annealing at 72°C for 30 s, and elongation at 72°C for 1 min. The annealing temperature was reduced by 1°C in each of the first eight cycles. Then followed 30 cycles of denaturation at 95°C for 1 min followed by annealing at 65°C for 30 s and elongation at 72°C for 1 min. There was a final elongation step at 72°C for 2 min. This PCR product, named GPDEREADH, was gel purified and cloned into pCR2.1-TOPO (Invitrogen Corp., Carlsbad, CA), creating pUTK408. The identity of the insert was confirmed by DNA sequencing.

GPDEREADH was cloned into pUTK401 to create pUTK407. To accomplish this, the GPD/ADH1 promoter region was removed from pUTK401 by double digestion with BamHI and SpeI and subsequent gel purification of the vector. Plasmid pUTK408 was similarly digested to excise GPDEREADH. A ligation of 1:10 vector pUTK401-insert GPDEREADH was performed using standard methods (27). The resulting ligand was electroporated into TransforMax EC100 electrocompetent *E. coli* cells (Epicentre, Madison, WI) and spread on LB plates with 100 μg ampicillin/ml.

S. cerevisiae hER containing hER-α in the chromosome was cotransformed with plasmids pUTK404 and pUTK407 to create strain BLYES (Fig. 1). Transformants were selected on YMM (leu^-, ura^-).

Bioluminescence estrogen assay. Strain BLYES was grown in YMM (leu^-, ura^-) overnight at 30°C and 200 rpm shaking to an approximate optical density at 600 nm (OD_{600}) of 1.0. Cells were centrifuged and resuspended in fresh YMM (leu^-, ura^-) to an OD_{600} of 1.0. Two hundred microliters was transferred to each well of a black 96-well Microfluor microtiter plate (Dynex Technologies, Chantilly, VA). Appropriate dilutions of test chemicals were added to each well. Bioluminescence was measured every 60 min for 12 h in a Perkin-Elmer Victor^2 multilabel counter with an integration time of 1 s/well.

Colorimetric estrogen assay. The YES assay was performed as described previously (28). β-Galactosidase activity was measured at 540 nm using the chromogenic substrate CPRG and normalized to growth rate via concurrent OD_{600} measurements.

EC_{50} calculations. For each chemical, bioluminescence or β-galactosidase activity (OD_{540}) versus the log of chemical concentration was plotted. A linear regression was determined using three or four points falling on the linear portion of the curve. Each 50% effective concentration (EC_{50}) (*x* axis) was calculated using the linear regression formula and the midpoint *y* value of each dose-response curve. For the 17β-estradiol standards, the EC_{50} was calculated individually for seven assays. The mean and standard deviation was calculated from the seven EC_{50} values to determine the variability between assays.

RESULTS AND DISCUSSION

***S. cerevisiae* BLYES.** Using a genetic scheme similar to *S. cerevisiae* BLYEV (12), *S. cerevisiae* hER was cotransformed with plasmids pUTK404 and pUTK407. Tandem estrogen response elements were inserted between the two constitutive divergent promoters GPD and ADH1 on pUTK401 (12), regulating *luxA* and *luxB*, respectively (Fig. 1). The resulting estrogen reporter was designated *S. cerevisiae* BLYES.

One advantage of bioluminescence assays compared to colorimetric assays is speed. When the BLYES strain was exposed to 2.8×10^{-9} M 17β-estradiol, quantifiable bioluminescence was observed in 60 min (Fig. 3). By 6 h, the assay reached a maximum bioluminescence and a lower limit of detection of 4.9×10^{-11} M (defined as twofold over background). In contrast, the colorimetric assay required 3 days before a response was measured and, for target compounds with low estrogenicity, 5 days of incubation were required for detection of the estrogenic response (18, 19, 25, 28). Although the incubation

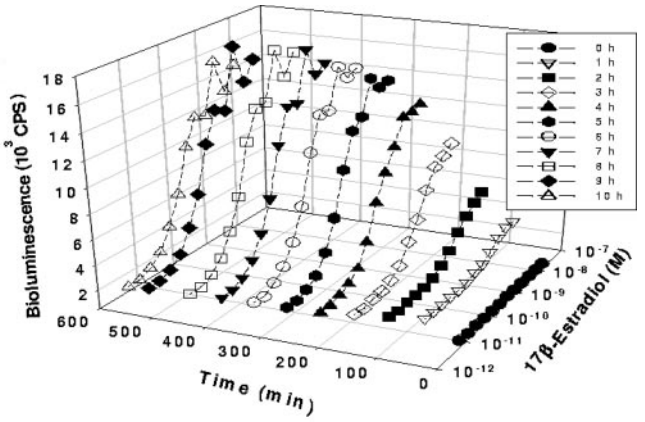

FIG. 3. Three-dimensional plot of bioluminescence versus time for 17β-estradiol. Initial bioluminescence was observed in as little as 60 min for 2.8×10^{-9} M and reached a maximum at approximately 360 min. CPS, counts per second.

time needed to measure estrogenic responses differed between BLYES and YES, the measured responses to 1.2×10^{-8} M through 5.6×10^{-12} M 17β-estradiol were similar for both strains (Fig. 4). This result was not unexpected, as the same host yeast strain containing the human estrogen receptor protein gene was used for both bioreporter constructs.

Bioluminescent dose-response profiles for specific estrogenic chemicals. Additional comparison of the two bioreporters was performed by challenging BLYES and YES with nine chemical compounds known to induce a range of estrogenic responses (Table 3). In these experiments, a series of six 1:10 dilutions of 17β-estradiol (ranging from 1.2×10^{-8} to 1.2×10^{-13} M) were used to generate the standard curves. Data for the bioluminescence and colorimetric assays were collected at 6 h and 3 days, respectively. The reproducibility of the bioreporter assays was determined by calculating the EC_{50} of 17β-estradiol from seven independent experiments. The calculated EC_{50} values from the colorimetric and bioluminescence assays

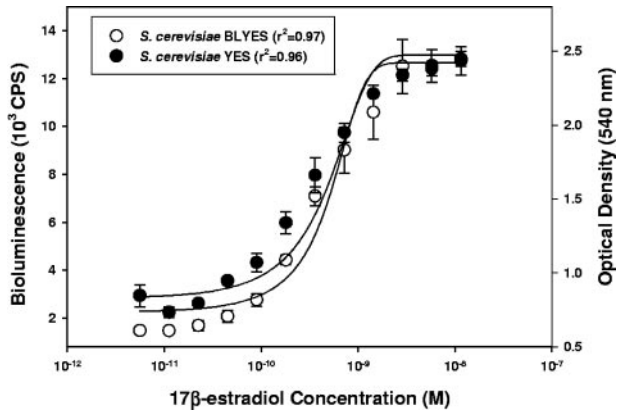

FIG. 4. EC_{50} dose-response profile of 17β-estradiol using *S. cerevisiae* BLYES (open circles) and *S. cerevisiae* hER (closed circles) reporter strains. The lower and upper limits of detection for each assay with 17β-estradiol were approximately 4.5×10^{-11} and 2.8×10^{-9} M, respectively.

TABLE 3. Comparison of EC_{50} values obtained by the colorimetric and bioluminescent yeast estrogenic assays using six-point 17β-estradiol standard curves

Chemical	EC_{50} (M)	
	Colorimetric assay	Bioluminescent assay
17β-Estradiol	$(4.4 \pm 1.1) \times 10^{-10}$ ($n = 7$ assays)	$(2.4 \pm 1.0) \times 10^{-10}$ ($n = 7$ assays)
17α-Estradiol	7.7×10^{-10}	4.3×10^{-10}
Estrone	1.8×10^{-9}	5.5×10^{-10}
17α-Ethynylestradiol	1.7×10^{-10}	2.5×10^{-11}
DES	7.3×10^{-12}	5.3×10^{-12}
4,4′-Cyclohexlidene bisphenol	7.7×10^{-7}	7.3×10^{-7}
Bisphenol A	4.9×10^{-6}	2.8×10^{-6}
Nonylphenol	NR[a]	1.7×10^{-5}
4-Andostrenedione	0.005	NR

[a] NR, no response.

were $(4.4 \pm 1.1) \times 10^{-10}$ and $(2.4 \pm 1.0) \times 10^{-10}$ M. These values are in agreement with previously published data (2, 9).

Seven chemicals tested were moderately to strongly estrogenic (8, 32). The EC_{50} values obtained by the two assays were very similar for 17β-estradiol, 17α-estradiol, DES, 4,4′-cyclohexlidene bisphenol, and bisphenol A, but they differed slightly for estrone and 17α-ethinylestradiol (Table 3). The EC_{50} values for these seven estrogenic compounds calculated from the bioluminescence assay were plotted against the EC_{50} values calculated from the colorimetric assay (Fig. 5). A linear regression analysis resulted in a slope of 0.99 and a coefficient of determination (r^2) of 0.99 and confirmed that strain BLYES provides data similar to strain YES for moderately to strongly estrogenic chemicals.

Two other chemicals tested, *p*-nonylphenol and 4-andostrenedione, resulted in different estrogenic responses between the two assays (Table 3). In the BLYES assay, nonylphenol induced a weak estrogenic response in the range consistent with the literature (9). However, in the colorimetric YES assay, nonylphenol reduced cell growth by 90% in the estrogenic concentration ranges and thus resulted in no estrogenic response. The difference in response between the BLYES and YES assays to *p*-nonylphenol suggests that the BLYES assay, which is a resting cell assay, is more immune to toxic effects than the YES assay, which is a growing cell assay. 4-Andostrenedione was included as a negative control. The negative response of the BLYES assay to 4-andostrenedione contrasts to the very weak response (more than a million-fold less estrogenic than 17β-estradiol) seen in the YES assay. Again, this difference may be due to the length of the incubation period for each assay. The 4-andostrenedione may be transformed over the 3-day incubation period of the YES assay, thus giving a false-positive result.

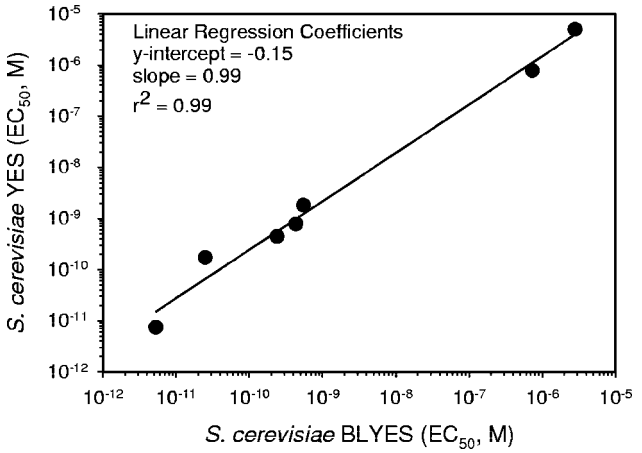

FIG. 5. Linear regression analysis of EC_{50} values determined by the bioluminescence and colorimetric assays. EC_{50} values are listed in Table 3. A coefficient of determination of 0.99 was achieved.

Conclusions. The environmental deposition of natural, pharmaceutical, and synthetic chemicals with estrogenic activities is associated with numerous human and wildlife physiological disorders, prompting the development of various assays to screen for estrogenic potencies (1). As a model towards demonstrating the applicability and inherent advantages of self-bioluminescent yeast bioreporters, a *lux*-based assay for environmental estrogens was developed and functionally compared to the established *lacZ*-based YES assay (26). Although proven effective for the in vitro determination of estrogenic activity, the YES incubation time of 3 to 5 days is impractical when considering the thousands of chemicals requiring screening. In contrast, the BLYES assay demonstrated a response time of <6 h for each chemical tested. In addition, the BLYES assay had the same or better sensitivity to the test chemicals as the YES assay.

Although the rapidity of the BLYES assay has been significantly improved compared to the YES assay, other potential limitations still exist. As demonstrated with the YES assay, the yeast cell wall and transport system can selectively decrease a particular chemical's potency or remain fully impermeable to it (1). Yeast-based assays have been criticized for their inability to differentiate between estrogen agonists and antagonists (4). However, Beresford and coworkers (2) demonstrated that these issues can be overcome or diminished through careful experimental design. However, we agree with their overall conclusion that a single assay system coherently functional for the thousands of chemicals requiring screening is unrealistic. Rather, a suite of assays will likely be needed.

The incorporation of the bacterial *lux* cassette under the control of estrogen response elements into *S. cerevisiae* hER will allow high-throughput screening of chemicals as required by the Food Quality Protection Act (Public Law 104-170). Further, *S. cerevisiae* BLYES, when combined with appropriate photodetection technology, can be used for remote, near-

real-time monitoring of our nation's waterways for endocrine-disrupting activity (3, 21).

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