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pYES with the substrate RGP for the detection of estrogen active compounds in sewage

Subject

The planar yeast estrogen screen (pYES) for the detection of estrogen active compounds (EAC) utilizes a genetically modified yeast strain of *S. cerevisiae* containing the DNA sequence of the human estrogen receptor (hER) and a reporter gene encoding for β-galactosidase. pYES with the substrate 4-methylumbelliferyl-β-D-galactopyranoside releasing blue fluorescing 4-methylumbelliferone (MU) after enzymatic cleavage was already deployed [1-6]. Since environmental samples often contain contaminants showing native blue fluorescence, the detection of MU can be interfered. Therefore, the substrate resorufin-β-D-galactopyranoside (RGP) was introduced, showing the orange fluorescence of enzymatically released resorufin in the HPTLC zone as positive signal for the estrogenic activity of a substance. The developed RGP-pYES was applied on extracts of influent and effluent samples of a waste water treatment plant. Moreover, the sewage samples were incubated with β-glucuronidase to detect possibly conjugated EAC.

Sample extraction

Spiked water samples (2-20 ng/L, 17α-ethinylestradiol **EE2** and 17β-estradiol **E2**) and sewage samples (influent and effluent) were extracted with TBME, and the separated organic phase was evaporated. The residue was re-dissolved in ethanol (concentration factor of 150). Additionally, sewage samples were incubated with β-glucuronidase (16 h) before extraction.

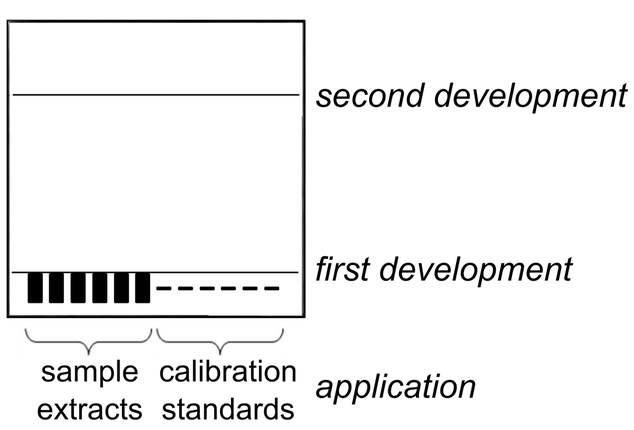
pYES

1. HPTLC

plate pretreatment
- adjustment to pH 6.5
- solution of $NaHCO_3$, pH 6.4

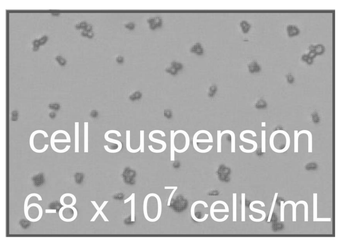
application
- extracts: 5x10 mm areas
- standards: 5-mm bands

chromatography

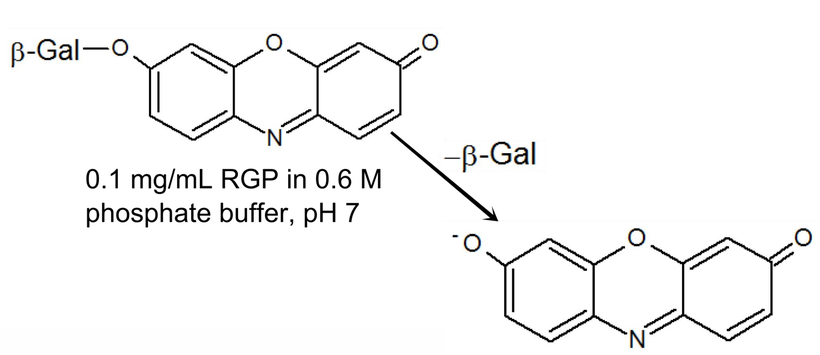


2. bioassay

yeast incubation
- 4 h, 30°C
- rel. humidity ~100%



substrate incubation
- 30 min, 37°C (3x)
- rel. humidity ~100%



3. documentation

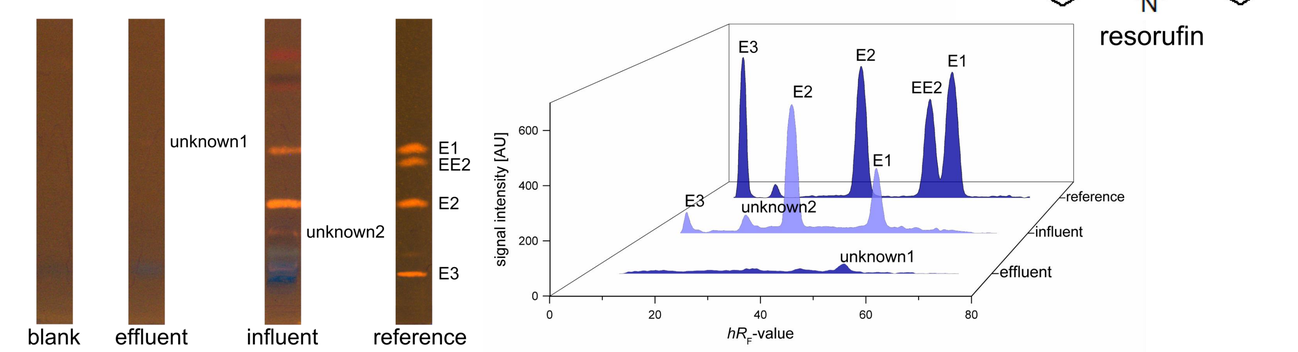


Plate images under UV 254 nm illumination (A) and the corresponding 3D densitograms (B) of the fluorescence scan at 550/>580 nm (E3 5 ng/zone, E2 100 pg/zone, EE2 100 pg/zone, E1 1 ng/zone).

Results & discussion

RGP, releasing orange fluorescent resorufin as positive signal for estrogenic activity, was shown to be a **suitable substrate** for pYES. The RGP-pYES provides the detection of EAC **without interfering signals** due to native fluorescences of sample contaminants.

The limits of detection (**LOD**) were 3 and 4 pg/zone, the limits of quantitation (**LOQ**) 5 and 8 pg/zone for E2 and EE2, respectively. **Recoveries** from spiked water samples were **close to 100%** for both E2 and EE2.

After extraction of the sewage samples and investigation by pYES, one unknown EAC in the effluent (**unknown1**) and four EAC (**unknown2**, **E2** ~ 17 ng/L, estriol **E3**, estrone **E1**) in the influent were clearly detected.

After incubation of the **influent** sewage sample with β-glucuronidase, the **E1 signal** and the **unknown2 signal** increased by about **25%** and **100%**, respectively (fig. 1).

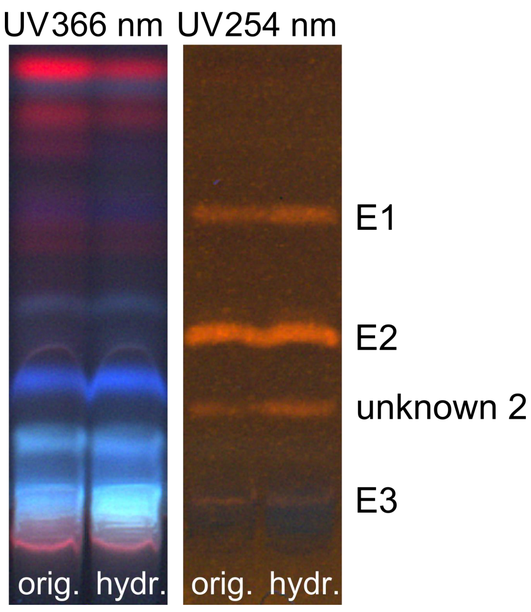


Figure 1: tracks of original and hydrolyzed influent extracts **left** after chromatography **right** after pYES

References

[1] S. Buchinger et al., Anal. Chem. 85 (2013) 7248-7256
[2] D. Spira et al., J. Planar Chromatogr. 26 (2013) 395-401
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[4] I. Klingelhöfer & G.E. Morlock, J. Chromatogr. A 1360 (2014) 288-295
[5] G.E. Morlock & I. Klingelhöfer, Anal. Chem. 86 (2014) 8289-8295
[6] I. Klingelhöfer & G.E. Morlock, Anal Chem. 87 (2015) 11098-11104

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