Science of the Total Environment 605–606 (2017) 507–513

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Separation and identification of hormone-active compounds using a combination of chromatographic separation and yeast-based reporter assay

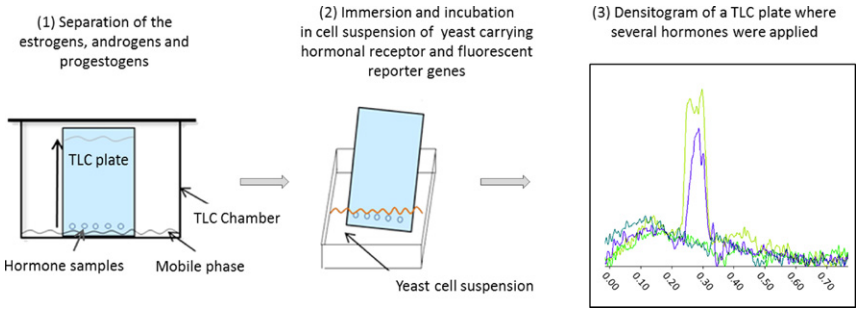
Alexandre Chamas [a], Ha Thi Minh Pham [a,b], Martin Jähne [c], Karina Hettwer [c], Linda Gehrmann [d], Jochen Tuerk [d], Steffen Uhlig [c], Kirsten Simon [e], Kim Baronian [f], Gotthard Kunze [a,*]

[a] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, D-06466 Gatersleben, Germany
[b] Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam
[c] QuoData GmbH, Prellerstr. 14, D-01309 Dresden, Germany
[d] Institut für Energie- und Umwelttechnik e. V. (IUTA, Institute of Energy and Environmental Technology), Bliersheimer Str. 58-60, D-47229 Duisburg, Germany
[e] New diagnostics GmbH, Moosstr. 92c, D-85356 Freising, Germany
[f] School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand

HIGHLIGHTS

- A combination of thin-layer chromatography and yeast-based assay is presented.
- This assay can detect picogram amounts of estrogens, androgens and progestogens.
- Environmental samples were successfully assayed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 April 2017
Received in revised form 9 June 2017
Accepted 9 June 2017
Available online 30 June 2017

Editor: D. Barcelo

Keywords:
Steroid hormones
Thin-layer chromatography
Yeast assay
Fluorescence
Wastewater samples

ABSTRACT

Arxula adeninivorans-based yeast cell assays for the detection of steroid hormones demonstrated their efficiency for the determination of total hormone activity in a variety of samples using a microtiter plate format. In this study, a preliminary chromatographic separation using thin-layer chromatography plates is introduced in order to allow a rapid identification of the compounds responsible for this hormonal activity. The yeast whole cell assay can then be performed on the plate, producing a detectable signal where a steroid hormone is present. Simultaneous detection of estrogens, progestogens and androgens on the same plate in the picogram range was achieved, while keeping the assay as simple and affordable as possible. The assay requires a single incubation of the thin-layer chromatography plate and the detection of reporter protein production can be performed by fluorescence scanning of the plate at different wavelengths. The chromatographic separation allows the separation of several estrogens, androgens and progestogens, thus making its application for 'real world' samples very useful. In this work, different water-based samples from environmental origins were used to demonstrate the capacity of this new bioassay. Trials showed that most samples, with the exception of complex samples such as wastewater influent, can be assayed.

* Corresponding author at: Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, D-06466 Gatersleben, Saxony-Anhalt, Germany.
E-mail address: kunzeg@ipk-gatersleben.de (G. Kunze).

http://dx.doi.org/10.1016/j.scitotenv.2017.06.077
0048-9697/

1. Introduction

Yeast-based bioassays, which can detect the biological activity of many compounds in the environment, play an important role in the field of bioanalytics. A significant proportion of these bioassays are dedicated to the detection of steroid hormones (Adeniran et al., 2015) as these can identify endocrine disrupting compounds (EDCs), compounds which can bind to hormone receptors and disturb the vertebrate endocrine system (Kabir et al., 2015; Casals-Casas and Desvergnes, 2011). There are yeast-based bioassays designed to detect estrogens (Kaiser et al., 2010; Sanseverino et al., 2008), progestogens (Viswanath et al., 2008; Chamas et al., 2015), glucocorticoids (Bovee et al., 2011; Pham et al., 2016), androgens (Bhattacharjee and Khurana, 2014; Gerlach et al., 2014) and more recently, for the simultaneous detection of several hormone classes (Chamas et al., 2017). Most of these bioassays combine the production of recombinant human hormone receptors and use enzymes or fluorescent reporter proteins to produce a quantitative signal.

Contrary to standard analytical detection methods which give the exact composition of a sample, yeast-based bioassays quantify the total hormonal activity of a sample and therefore assess the biological impact a sample can have. Yeast-based bioassays are thus often used as a pre-screening procedure to give an overview of a samples hormonal activity before analytical detection methods are used to determine which compounds are present in the sample and identify potential EDCs. In an effort to combine the bioassay and analytical approaches, recent studies have introduced a bioautography detection method using thin-layer chromatography and cells from the yeast-based estrogen screen (YES) assay (Mueller et al., 2004; Spira et al., 2013). In these tests, an unknown sample is firstly separated on a thin-layer chromatography plate together with control molecules with known estrogenic activity. After separation, the plate is sprayed with an engineered yeast cell suspension producing a β-galactosidase when an estrogenic substance binds to the recombinant human estrogen receptor. The presence of this enzyme is then detected by spraying the plate again with a chromogenic or fluorescence-producing substrate. With this planar-YES assay, compounds with estrogenic activity have been detected in various environmental samples (Klingelhöfer and Morlock, 2015; Buchinger et al., 2013), cosmetic products (Buchinger et al., 2013) and food samples (Klingelhöfer and Morlock, 2014). Detection of 17β-estradiol in the femtogram range was reported in environment samples, thus enabling ultratrace level determination. A great advantage of TLC based assays over regular microtiter plate based assays is that prior separation of a complex sample during thin-layer chromatography will remove compounds that could be toxic to yeast. Another advantage of the planar-YES assay is that it requires less than 24 hours incubation to obtain a signal after chromatographic separation whereas the regular YES assay requires incubation for 3 to 5 days to obtain an unequivocal result (Routledge and Sumpter, 1996).

To obtain sharp bands and very low detection limits, the latest optimizations of the planar-YES assay employs high performance thin layer chromatography plate coatings such as Reverse phase RP-18W (Klingelhöfer and Morlock, 2014) and fluorescence-producing substrate 4-methylumbelliferyl-b-D-galactopyranoside (MUG) (Buchinger et al., 2013). These improvements make the planar-YES assay of comparable performance to the established analytical methods, offering determination of a samples biological activity at low concentrations as well as identification of the compounds. However, the assay is more complex and more expensive than previous assays which may reduce the attractiveness of this analytical/biological detection method.

In an effort to incease simplicity in preference to low sensitivity and a broad detection range, we designed a bioassay combining analytical separation with unmodified silica gel thin-layer chromatography plates and detection without addition of an enzyme substrate. To achieve the latter goal, fluorescent reporter proteins were used, obviating the need for substrate. Moreover, ideal EDC detection should not only detect estrogenic molecules but also other EDCs such as progestogens and androgens. Therefore our aim was to design a bioassay able to determine in one analysis which endocrine activity is present in a sample and which compounds are responsible for it. This assay, to our knowledge, is the first to possess these characteristics and could be developed as an assay for other molecules.

Modified *Arxula adeninivorans* strains were used as the biocomponent in the assay. These strains, described in a recently published research article (Chamas et al., 2017), produce a recombinant human progesterone, androgen or estrogen receptor, each in combination with the production of a fluorescent reporter protein, CFP, GFP or DsRed2 respectively. In the microtiter plate assay, these strains were mixed and could then, once incubated with a mixture of estrogens, androgens and progestogens, determine which particular hormone activity is present in the sample. It was then decided to test if this yeast strain mix can be combined with thin-layer chromatography and after three fluorescence scans of a plate, determine which hormone activities are present in a sample. To achieve this, the bioassay should realise these objectives: (1) all three *A. adeninivorans* strains should be able to interact with their target hormone on a thin-layer chromatography plate, (2) the fluorescence emitted from this interaction should be detectable with a plate scanner, (3) the three yeast strains, when mixed, should give easily distinguishable signals for each of the three hormonal types, (4) the development conditions for the assay should be uncomplicated.

2. Material and methods

2.1. Chemicals

Estrone (E1), 17β-estradiol (E2), estriol (E3), progesterone (P), medroxyprogesterone acetate (MP), mifepristone (M), nandrolone (N), trenbolone (T), 5α-dihydrotestosterone (DHT) were all purchased from Sigma-Aldrich (Steinheim, Germany). All hormones were solubilized in ethanol at a stock concentration of 1 g/l. Acetone, dichloromethane and cyclohexane were all also purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Strains and cultivation conditions

The yeast strains used in this work, (*A. adeninivorans* G1212/YRC102-hPR-CFP, G1212/YRC102-hAR-GFP and G1212/YRC102-hER-DsRed2), are described in a previous publication (Chamas et al., 2017). Each strain was cultivated for 24 h in yeast minimal media supplemented with glucose (Yeast Minimal Medium [YMM]-glucose) at 30 °C until the $OD_{620\ nm}$ reached approximately 3. The cells were pelleted by centrifugation at 4000 × *g* for 10 min, the pellet was resuspended in an equal volume of fresh YMM-maltose and the three cell suspensions were mixed together to create *A. adeninivorans* G1212/YRC102-hHR-fluo at a final total $OD_{620\ nm}$ of 3.

2.3. Thin-layer chromatography procedure

100 × 200 mm polyester sheets precoated with 0.2 mm silica gel and without fluorescent markers (Macherey-Nagel, Düren, Germany) were used for all thin-layer chromatography experiments. For automated application of samples, the desired volumes were applied using an automatic TLC Sampler 4 (CAMAG, Muttenz, Switzerland) as 6 mm bands, 8 mm from the bottom of the plate. The application zones were allowed to dry at room temperature for 5 min and the plate was inserted in a TLC vertical development chamber containing dichloromethane:cyclohexane:acetone (60:35:5, v/v/v). Once the solvent migrated to within 85 mm from the bottom of the plate, the plate was removed from the developing chamber and allowed to dry at room temperature for 30 min. For manual sample application, the desired volume was applied with a glass capillary at 10 mm from the bottom of the plate. After drying for 5 min at room temperature, the plate was focused two times with methanol until the solvent