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Short communication

Calorimetric detection of the toxic effect of androgens on fission yeast

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ABSTRACT

Calorimetry is evaluated for study of the toxic effect of environmental androgens on *Schizosaccharomyces pombe* cells. The results indicate that androstendione, androstandiendione and dehydrotestosterone inhibited *S. pombe* heat production rate. Although, the turbidimetric method showed that testosterone (TS) had no influence on growth of *S. pombe*, calorimetry revealed that there was a shift in growth period in samples with TS.

1. Introduction

Contamination of the environment with toxic compounds is an increasing problem. Chemicals can disturb hormone signalling and homeostasis in various organisms [1,2]. These endocrine disrupting compounds (EDCs) have been reported to cause developmental abnormalities and reproductive dysfunctions in vertebrates [3–5,2]. EDCs include environmental androgens, compounds generated by bacterial biodegradation of naturally occurring phytosterols. These androgens cause female masculinization in several species of fish [6–9].

Effects of environmental androgens have been studied mainly in vertebrates such as mosquitofish, trout and three-spined stickleback[10–12]. However, there is a need for a simple, unicellular eucaryote model to facilitate investigations of mechanism(s) of environmental androgens toxic effect on eucaryote cells. Our previous work reported that fission yeast can also be a useful model for androgen detoxification because these organisms can transform toxic androgens (androstendione, AD) to less toxic compound (testosterone, TS) [13]. Using *Schizosaccharomyces pombe* as a model organism has several advantages [14–16], but there is a need for a method for detection of the effects of androgens on fission yeast. Methods commonly applied for measurement of yeast growth rate include turbidimetry and determination of dry mass, but nonviable cells are also measured. Furthermore, these methods are not continuous. Therefore, there is a need for a method to measure viable cells in a small amount of medium and to determine the cell metabolic state.

Calorimetry is a useful technique for measuring the growth rates of microorganisms in the presence of anti-microbial agents, tea tree or essential oils, and xenobiotic compounds [17–21]. There are also data on application of calorimetry for growth rate analysis of bakers yeast [22]. The aim of this work is to determine whether calorimetry is a suitable method for determination of toxic effects of environmental androgens, such as AD and androstandiendione (ADD) and their less toxic analogues – testosterone and dehydrotestosterone (DHTS) on fission yeast cells.

2. Experimental

2.1. Chemicals

Androgens (AD, ADD, TS, DHTS) and 96% ethanol were from Sigma (Steinheim, Germany). The FUN-1 dye was purchased from Molecular Probes (Eugene, USA). All chemicals used were high purity reagents.

2.2. Microorganism and culture conditions

Fission yeast *S. pombe* 972h^- haploid strain from the collection of Dr. U. Leupold, University of Bern was used. All studies were carried out at 28 °C on YEL (Liquid Yeast Extract Medium) as descried elsewhere [23,24]. Briefly, 48 h *S. pombe* cultures on YEA (Yeast Extract Agar) slants were used to inoculate 20 mL YEL in conical flasks and

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incubated on a rotary shaker overnight. After the incubation, *S. pombe* cells were diluted in YEL medium to 1×10^7 cells mL^{-1}.

2.3. S. pombe Bioscreen C culture

S. pombe growth was determined with turbidity measurements on Bioscreen C Analyser System (Labsystems, Finland) at 28 °C. Each well of the Bioscreen C microtiter plate was filled with 300 μL of *S. pombe* cells suspension (1×10^7 cells mL^{-1}) and supplemented with androgens (dissolved in ethanol) to a final concentration of 0.5 g L^{-1}. Ethanol was added to the control cultures. Each sample was repeated ten times. The Bioscreen C was programmed to measure optical density (OD) automatically every 3 h of incubation at a wavelength of 600 nm. Growth curves are presented as OD plotted against time.

2.4. Calorimetry

All calorimetric experiments with *S. pombe* were done as described earlier for other microorganisms [19,21,25]. Briefly, the experiments were conducted at 28 °C with Micro DSC III (Setaram, France), with sterilized, stainless batch vessels with a total volume of 1000 μL. The sample vial contained 300 μL of *S. pombe* suspension (1×10^7 cells mL^{-1}) in YEL medium (with or without androgens) and the reference vial contained 300 μL of sterilized YEL medium with ethanol. Androgens (dissolved in ethanol) were added to a final concentration of 0.5 g L^{-1}. To the control cultures ethanol was added. Recording was started 30 min after the sample vials were closed and placed in the calorimetric block.

2.5. S. pombe morphology and viability

The morphology and viability of *S. pombe* cells were measured after the calorimetric and turbidity studies. Viability was determined with FUN-1 staining and image processing on CLSM (Confocal Laser Scanning Microscopy) as described earlier [26]. Cell counts were estimated by counting in Thoma chamber.

2.6. Statistical analysis

Normality of distribution was verified with Shapiro-Wilk's *W* test. To estimate significance of differences, one-way ANOVA (with NIR test) or Student's *t*-test was used.

3. Results

3.1. S. pombe growth

Comparison of the results obtained by Bioscreen C and calorimetry is shown in Fig. 1.

The maximum heat rate was detected at 14.5 h, in the exponential growth phase as shown by Bioscreen C method. After 34 h of incubation, the heat rate was at the baseline (with just media in both ampoules), suggesting that *S. pombe* reached a stationary phase. The turbidimetric method showed that *S. pombe* growth reached a stationary phase after 24 h and the OD values did not change up to 72 h. Both methods indicated the transition of cells to a stationary phase, but in comparison with turbidimetry, calorimetry detected changes in growth rate earlier.

3.2. Detection of toxic effects of androgens on S. pombe

The results of calorimetric experiments in media supplemented with different androgens are shown on Fig. 2A.

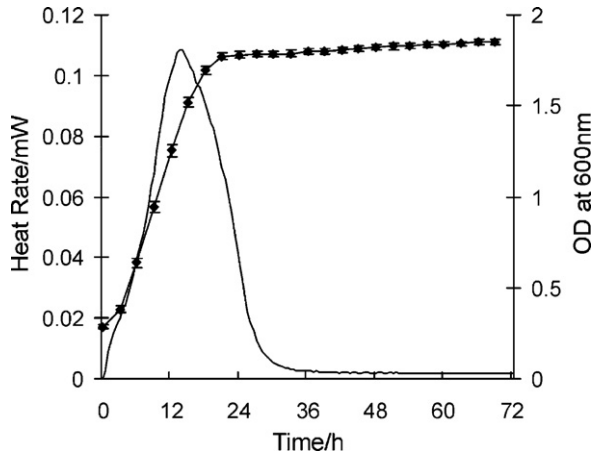

Fig. 1. *S. pombe* growth measured by calorimetry (solid line) and turbidity (line with rhombus).

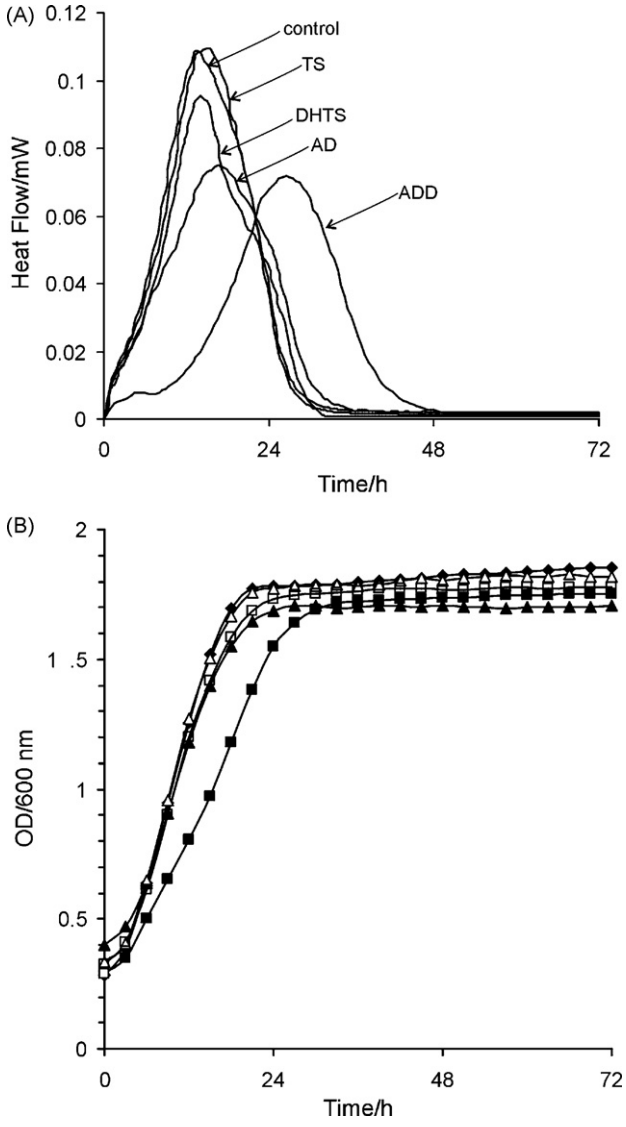

Fig. 2. *S. pombe* growth in the presence of androgens. (A) Heat production rate. (B) Turbidimetric growth measurement. Control without androgens (black rhombus), AD (black triangles), TS (white triangles), ADD (black squares) and DHTS (white squares).

AD, ADD and DHTS inhibited *S. pombe* heat production rate. The peak heights were significantly lower in comparison to the control and samples incubated with TS.

The data obtained with the turbidimetric method are given in Fig. 2B. Introduction of AD, TS and DHTS to the cultures caused a significant ($p < 0.005$) increase of OD at 0 h in comparison to the control. This result was probably an effect of low solubility of androgens in the medium, but is confusing because it suggests the number of cells differ between samples. No differences at 0 h were observed by calorimetry (Fig. 2A).

Both methods indicate that AD and ADD caused *S. pombe* growth retardation (Fig. 2). ADD inhibited the heat rate up to 8.5 h and AD and ADD caused shifts in *S. pombe* growth periods. Statistical analysis of the data measured by turbidimetry also revealed that AD and ADD significantly inhibited growth (from 3 h to the end of incubation; $p < 0.005$, in comparison to the control). The influence of DHTS was strongly marked in the calorimetric results by a lower heat rate. In the turbidimetric method, statistically significant differences between control and samples with DHTS were noted between 15 h and 21 h and from 45 h to the end of the incubation.

Although the statistical analysis of the OD values suggested that TS had no influence on *S. pombe* cells, calorimetry revealed a shift in growth period, the maximum heat rate was delayed by 2 h (Fig. 2A).

3.3. Incubation of S. pombe with androgens

The aim of this series of experiments was to determine whether the incubation of *S. pombe* with androgens in the calorimeter and Bioscreen C affected the cells' condition, number and viability in a similar manner. After incubation in the calorimeter and Bioscreen C, the number of *S. pombe* cells and cell viability were not significantly different (Student's *t*-test) in adequate samples (Table 1).

Additionally, after incubation in the calorimeter and Bioscreen C, microscopic inspections were done (Figs. 3 and 4). In control samples from both, the cells did not reveal any morphology changes (Figs. 3E and 4E), but cells incubated with AD or ADD displayed similar morphology changes with aberrant swollen shapes (Figs. 3A, C and 4A, C, respectively). TS and DHTS had no influence on *S. pombe* morphology (Figs. 3B, D and 4B, D, respectively). The results obtained from microscopic inspections suggest that growth conditions were similar in both instruments.

4. Discussion

The maximum heat production rate was detected in the exponential phase of yeast growth. Similar results were reported for *E. coli* [20]. This observation is important because microorganisms exhibit the highest sensitivity toward toxicants in the exponential growth phase [20,27]. The results show that calorimetry is a convenient tool for estimation of growth rate of fission yeast. In the case of AD and ADD, the thermal profiles were delayed, but in the case of TS and DHTS no delay was observed. This finding is consistent with our previous results, where AD had more toxic effect on *S. pombe* than TS [13]. DHTS decreased the heat production rate, but there was no delay in thermal profile. Literature data suggest that when a compound shows a strong anti-microbial action, the initial number of viable cells is reduced and the thermal profile is delayed [18,28]. On the other hand, in filamentous fungus *Cunninghamella elegans* cultured in the presence of phenanthrene, no delay in thermal profile was observed, while the heat production rate was decreased [19]. Therefore, our results suggest that AD and ADD had both toxic and anti-microbial effects while DHTS had only toxic effect on yeast. This is consistent with the results on cell viability (see Table 1) and is a further argument in favour of calorimetry as a tool to study androgen toxicity in yeast.

Table 1
Comparison of cell count and viability of *S. pombe* after incubation in the calorimeter and Bioscreen C

	Calorimeter		Bioscreen C	
	Cell count (mL^{-1})	Viability (%)	Cell count (mL^{-1})	Viability (%)
Control	7.8×10^9	96.9 ± 2.5	8×10^9	96.5 ± 1.8
AD	9.1×10^8	62.4 ± 1.3	9.1×10^8	58.7 ± 6.8
TS	6.8×10^9	93.4 ± 1.2	7.1×10^9	90.1 ± 3.8
ADD	5.1×10^8	21.0 ± 9.0	5.3×10^8	21.5 ± 12.5
DHTS	2.6×10^9	83.1 ± 2.4	2.5×10^9	81.5 ± 1.8

For viability means ± S.D. are shown; cell count S.D. did not exceed 5% for all values, and for clarity of the table, S.D. values are not shown.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tca.2008.05.007.

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