Comparison of ergosterol to other methods for determinations of *Fusarium graminearum* biomass in water as a model system

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Summary

Ergosterol increased with visual estimations of biomass of *Fusarium graminearum* in water. Ergosterol is recommended for total fungi estimations in water.

Keywords: Ergosterol; Fungi; Biomass; Water

Introduction

Fungal contamination in water systems has been known for many years, and is receiving more attention (Kelley *et al.*, 2003). Fungal biomass determinations are problematic generally in terms representing quantities of fungi. Often total fungi using colony forming units (cfu) are employed where it is not known how many cfu form individual colonies. Dry weight determinations are used frequently with liquid growth media, although what this represents in terms of increases in numbers is unclear. A gossamer (loose pellet) growth form can be observed in enclosed vessels with dilute media. Visual inspection of water is effective to determine if it contains these pellets, although this would not be done by the consumers of bottled water consistently. Even less obvious biomass can occur. The fungi may pose a health risk to consumers especially from mycotoxin production. In addition, fungi appear to contribute to biofilm formation in water distribution systems.

Ergosterol (Fig. 1) is an effectively universal and exclusive fungal lipid which constitutes part of the cell membrane. It has been used for over 30 years to estimate fungi, and there is enough data in the scientific literature to conclude that it is effective. Sridhar and Barlocher (2000) used ergosterol to conclude that fungal production in streams is stimulated by inorganic N and P. The greater fungal biomass allowed increased production of invertebrates, and higher tropic levels, another potential source of problems from fungal contamination of water. CABI Bioscience and MUM are involved in an EU funded project on fungal contamination of bottled drinking water (COMBOW), and MUM is undertaking an EU project on the microbiological safety of water in distributions systems (SAFER) where the determination of fungal biomass in water is necessary. Ergosterol from *Fusarium graminearum* grown in water was determined and compared to other methods and the results are presented in the present report.

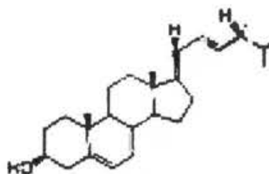

Figure 1. Chemical structure of ergosterol.

Material and Methods

F. graminearum (IMI 374111) was grown on malt agar at room temperature and a suspension of it was made in deionised water to a known concentration based on cfu. Deionised water (DI), and DI plus glucose (1 mg l^{-1}) were inoculated with the equivalent of 3 cfu in 1 ml DI. Uninoculated DI was the control. The flasks were shaken at 100 rpm for 14 days, filtered through 90 mm Whatman no. 1 and dried for 24h at 60 °C. Tap water was treated similarly, but filter papers (fp) were analysed wet.

Fp were weighed by standard laboratory equipment, and refluxed in methanol:ethanol (5:1v/v) with 10 % (w/v) potassium hydroxide. Filtrates were extracted with 60 ml petroleum ether, dried (rotary evaporation), and resuspended in 15 ml methanol and filtered through 0.45 μm Millipore filters. These were dried by air streams and

resuspended in 250 µl methanol. The dried petroleum ether fraction was redissolved in 2 ml of methanol and filtered through 0.45 µm with 3 washes of methanol. The methanol was evaporated and redissolved in 200 µl methanol. Samples were analysed by HPLC (Waters 600, 486 UV detector at 282 nm, 746 data module programmed to calculate ergosterol concentration). Column was a reversed phase Nova-pak C18 (3.9 × 150 mm). Solvent was HPLC methanol; flow rate 2 ml min^{-1}. Injection volume was usually 100 µl but lower volumes were also used.

Results

The dry weight data were very variable, and even negative data were obtained. Chromatograms of ergosterol from the fungus were devoid of many additional peaks. The VE and ergosterol concentrations are presented in Table 1.

Table 1. Ergosterol concentration and visual estimation growth (VE) of *F. graminearum* in water

Ergosterol [ng l^{-1}]		VE
Control DI (×4)		
	nd*	0
DI glucose		
	nd*	1
	60	2
	nd	1
	nd	2
DI		
	nd	1
	1240	3
	540	3
	600	2
Tap water		
	120	1
	720	2
	720	2
	840	2
	3680	3

*Injection volume less than 100 µl. nd = not detected.
0 = no growth; 1 = some growth; 2 = more growth; 3 = most growth.

Values ranged widely within each treatment. Ergosterol was not detected in samples where 1) VE = 0, and 2) VE = 1 or 2 on some occasions. Overall, ergosterol concentration increases with higher VE (Fig. 2).

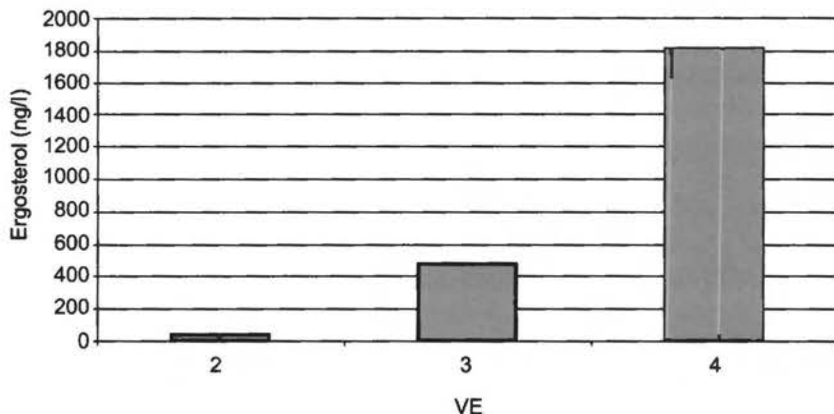

Figure 2. Ergosterol (ng/l) *versus* visual inspection (VE) (2, 3 and 4 = some, more, and most growth, respectively).

Discussion

The dry weight results were disregarded due to variability confirming that the mass was too low for conventional equipment. The HPLC peaks for ergosterol were separated from other material assisted by the purity of the sample growth medium (*i.e.* water). The variation in the results for each treatment logically reflects the use of cfu for inoculation where, for example, one or many conidia could act as the inoculum(a). It is a good experimental system for demonstrating this problem. It would be useful to detect ergosterol when VE = 0 but there was some biomass in terms of the predictive value of the method. However, in all cases where ergosterol was not detected and VE = 0, the injection volume was less than 100 μl which may have been too low. More work at these levels is required, for example, by measuring ergosterol periodically during growth. Non detection of ergosterol from flasks with VE > 0 is an anomaly probably related to manipulating and extracting low levels of biomass. VE are qualitative and subjective although adequate within such limitations. It is worth pointing out

that plating out methods would be capable of detecting a few conidia in water, whereas it is unlikely that the method described here could detect 1 or 2 spores (*ca.* 1 pg ergosterol conidia^{-1}). The apparent differences in values for each treatment *(e.g.* DI, tap water) is not the topic of this paper and is not discussed.

F. graminearum is one of the main mycotoxin producing fungi (Paterson *et al.*, 2003) was isolated occasionally from the US water distribution and could produce mycotoxins including zearalenone in water (Kelley *et al.*, 2003). A value for ergosterol in drinking water could be recommended above which remedial action would be required. In conclusion, ergosterol is recommended for total fungal estimations in water and other dilute systems.

Safety

Treat mycotoxigenic fungi as though they have produced mycotoxins. Adhere to national guidelines for dangerous chemicals.

Acknowledements

Work undertaken as part of the project detailed in Kelley *et al.* (2003).

References

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