CYTOTOXICITY ANALYSIS FROM INNER AIR SAMPLES

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Cytotoxicity tests using specialized cells, e.g. boar sperm or mammalian somatic cells, can be used as the toxicity sensor for indoor fungi and bacteria. In our work, samples collected from buildings with inner air problems were cultivated and the microbes were analysed using two toxicity tests basing on inhibition of 1) boar sperm motility, and 2) mammalian cell growth. With both methods we can get more information about the toxin producing microbes when they colonize buildings suffering from moisture problems.

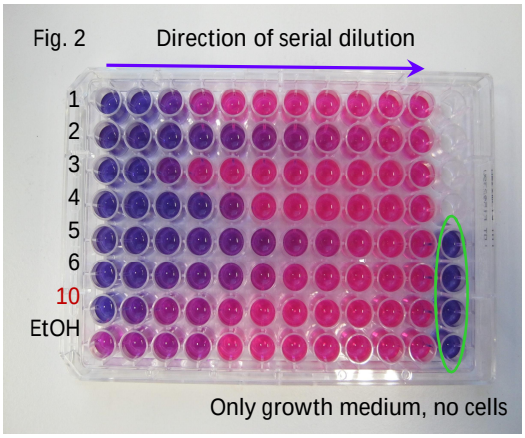


Fig. 2. Toxicity test with mammalian somatic cells

Baby hamster kidney (BHK-21) cells were incubated with microbial extracts in Dulbecco's modified Eagle's medium. After incubation, inhibition of BHK cell growth was assessed using the resazurin reduction assay (Bencsik et al., 2014). Ethanol was used as a negative control (last row). In rows 1 – 6 were extracts from toxic fungi previously isolated from houses with moisture problems. Colony 10 was one of the colonies originating from office air sample. BHK cells produced similar result profiles as murine neuroblastoma (MNA) cells used previously (Rasimus et al. 2012).

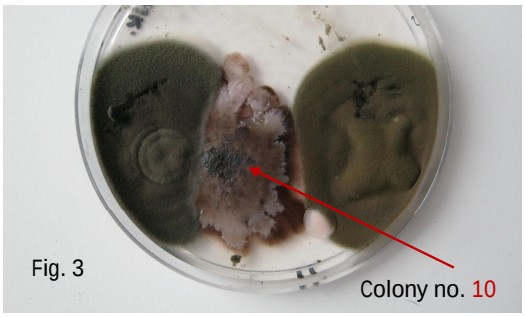


Fig. 3. Cultivation of samples on agar plates

Fungal growth on dichloran-glycerol (DG18) agar. Colony no. 10 proved to be toxic in both sperm and BHK-cell tests (Fig. 2). Inner air samples were grown on three agar plate types: for fungi i) malt extract (MEA) and ii) DG18, and for bacteria tryptone glucose yeast extract (TGY).

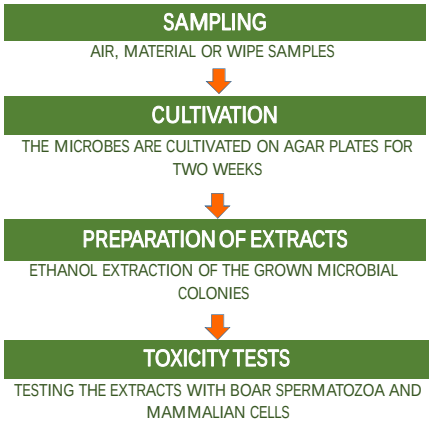


Fig. 1. Protocol chart for toxicity tests

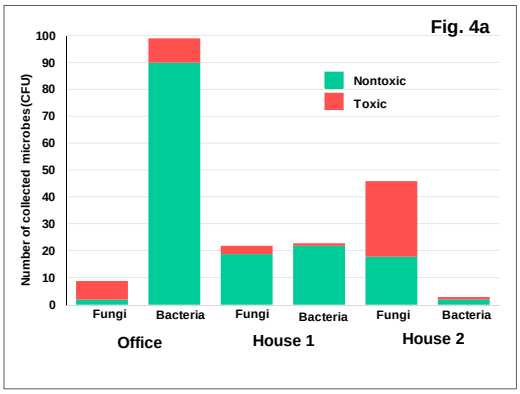
All grown colonies differing in their appearance were extracted with ethanol (10-20 mg cells per 0.2 ml), and the extracts were boiled.

In this work, air samples were collected with a 6-stage cascade impactor or by placing a filter cloth to window with replacement air coming through.

RESULTS AND CONCLUSIONS

More microbial toxins could be detected with mammalian somatic cells than with sperm test. Surprisingly few fungal toxins gave positive reaction in both assays. The advantage of both cytotoxicity tests is that they can identify toxic fungi even if they are not included in the official indicator list. They can also sense bacterial toxins.

Numerous toxic compounds producing by microbes in buildings can be detected with either sperm or mammalian cell test (or both). The only known microbial toxin, which cannot be detected using these tests, is ochratoxin.



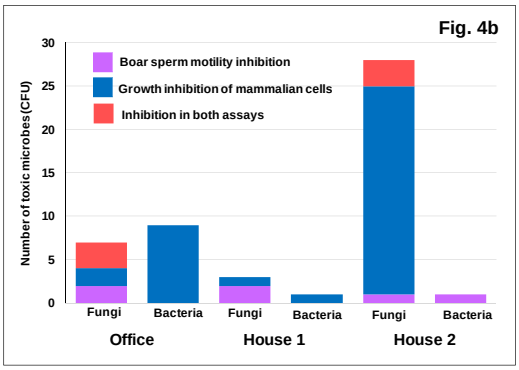


Fig. 4. a) The number of toxic and nontoxic fungi and bacteria (CFU, colony forming unit) received from indoor air or material samples collected from an office and two houses in Helsinki and Porvoo, Finland. All together 85 microbial colonies were tested from total 196 colonies grown on plates.

b) The number of toxic fungi and bacteria, extract of which caused inhibition of boar sperm motility, inhibition of cell growth of mammalian somatic cells , and inhibition in both assays.

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Literature

Bencsik et al. (2014) Toxins 6, 2857-2871.

Rasimus et al. (2012) Appl. Environ. Microbiol. 78, 3732 – 3743.