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Research output: scientific > Abstract

Flow cytometry to analyse *Penicillium expansum* spores at single spore level and impact of matrix, heat treatment and incubation temperature on spore outgrowth

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Penicillium expansum is a post-harvest plant pathogen that typically affects apples and pears, but also other fruits including citrus fruits, and cherries. Besides the economic loss resulting from infection of fruit with this mould, it also poses a health concern because of the production of the neurotoxin patulin.

Previous work showed that *P. expansum* is dominating mould in fruit smoothies that were treated with pulsed electric field to extend shelf life. Time to visual mould growth was affected by storage temperature but also varied across the different individual bottles studied possibly reflecting heterogeneity in spore outgrowth.

To study the outgrowth capacity of *P. expansum* spores in fruit juices, flow cytometry coupled to a cell sorter was exploited and performance of individual spores was analysed in either fruit juice or on fruit juice agar. Single or multiple spores of freshly harvested or glycerol (30%) containing stocks from the -80°C freezer were sorted on petri dishes or 48- or 96-wells plates filled with agar containing either malt extract pH 5.4 or pH 3.6, orange juice, apple juice or strawberry smoothie. Plates were incubated at either 7 or 22°C.

Using the above described approach we could establish that:

- Even after storage for 3 months, depending on the growth medium 94 -99% of the *P. expansum* spores stored at -80°C (with 30% glycerol) was able to grow out at a rate comparable to freshly prepared spores.
- Time to visual growth was mostly affected by temperature and only a limited effect of medium composition was observed.
- The effect of stress (freezing, heat and cold plasma treatment) on inactivation and heterogeneity in outgrowth of surviving mould spores could be quantified.

In conclusion, this method proved to be a highly efficient and useful method to study performance of individual mould spores on either agar plates or multi-well plates (liquid or agar medium) in a high throughput format. It allows to quantify impact of environmental conditions, food processing or antifungal compounds on outgrowth of individual fungal spores.