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Letter to the Editor

Alternative patulin pathway unproven

Dear Editor,

We read with interest in your journal that *Penicillium expansum* strains S3, S31 and S87 (Rharmitt et al., 2016) were considered negative for possession of the isoepoxydon dehydrogenase (*idh*) gene for patulin (PAT) production and positive for PAT production. This led to speculation that an alternative pathway was involved in patulin production. However, the authors did not provide the diode array (DA) UV spectra of the HPLC peaks assigned to PAT, which assist in confirming its identification. UV spectra could usefully be provided in papers when DA analysis has been performed especially for unusual results such as these: At least a statement to say whether the spectra were identical, or not, to the PAT standard is required.

One of the current authors (RRMP) reported for the first time the PCR test to assess mycotoxigenic fungi using the *idh* gene coupled with PAT detection by (a) TLC (Paterson et al., 2000; Paterson et al., 2003) and (b) HPLC UV (Paterson et al., 2003). *idh* negative and PAT positive strains were not detected. Similarly, Luque et al. (2011) did not detected this combination using micellar electrokinetic capillary electrophoresis, and HPLC-MS for PAT detection. The principle was employed in reverse transcription real-time PCR for *idh* (De Clercq et al., 2016).

The current authors found that a compound which has (a) the same retention time as PAT, but (b) a different UV spectrum using Ultra HPLC DA detection (UHPLC DAD) in a *Penicillium* strain (Fig. 1), during recent screening work to detect patulin from penicillia isolated from Tunisian apples. Hence, the strain was negative for PAT detection. This was confirmed by the strain being *idh* negative. Incidentally, the chromatogram for *P. expansum* (S13) in Rharmitt et al. (2016) had a co-eluting metabolite with PAT as deduced by the peak having a distinct shoulder. The UV spectrum of this sample would probably be different from pure PAT although this was unreported.

Perrone et al. (2017) recommended MS and NMR to establish correct ochratoxin A (OTA) production in species not associated conventionally with this mycotoxin. The questionable data were from HPLC-FLD (fluorescence detection) where other fluorescent compounds with the same retention time could be misidentified as OTA. Few laboratories may be able to afford MS and NMR and all methods need assessing on a cost-benefit basis to indicate what is suitable for most laboratories (Paterson et al., 2018). Rharmitt et al. (2016) employed HPLC DAD but the analytical potential of the technique was unfulfilled. Furthermore, Rharmitt et al. (2016) suggested the primers they employed may not have bound to the target DNA hence the negative *idh* result, which also undermines their premise of a different pathway for PAT.

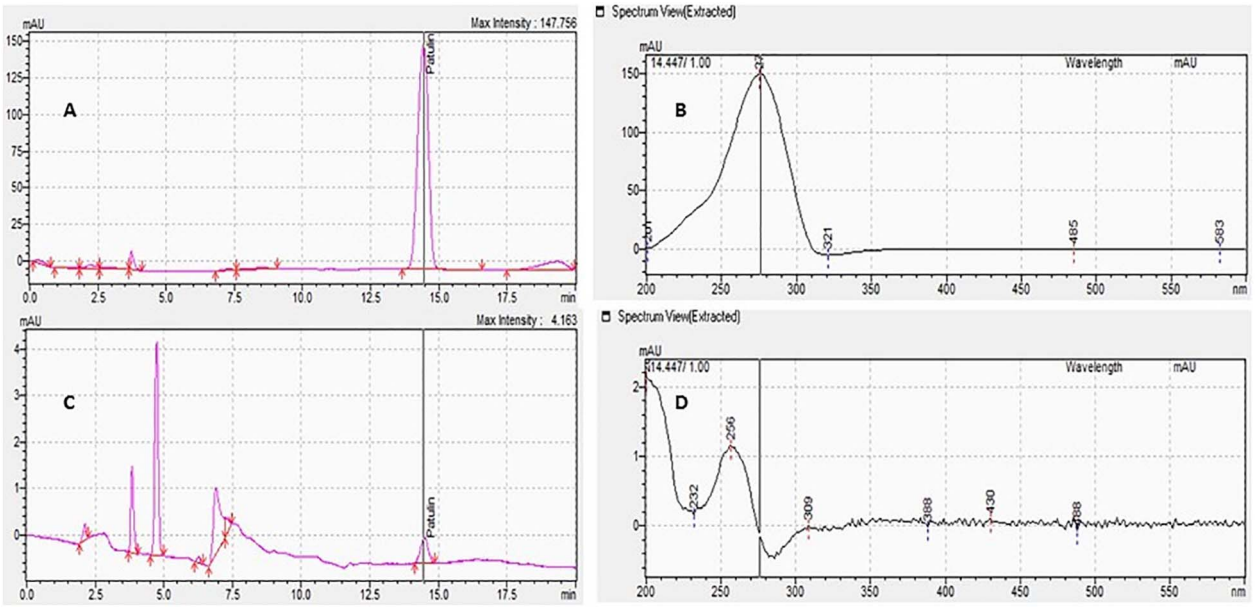

Fig. 1. UHPLC chromatograms and UV spectra for the (i) *idh* negative and patulin negative *Penicillium* strain (MUM 17.62) and (ii) patulin (PAT) standard. A, B are the chromatogram and UV spectrum of the PAT standard respectively. C is the false PAT positive chromatogram and D is the PAT negative UV spectrum for the strain.

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Researchers require circumspection when proposing changes to theories including those relating to metabolic pathways. It is essential to confirm that *idh* is the only route for PAT production in these mycotoxigenic fungi (a) to verify the PCR test based on the *idh* gene, (b) to develop biocontrol agents without the ability to produce PAT, (c) for chemotaxonomic purposes and (d) for genetic and physiological studies on PAT and secondary metabolism.

Stackebrandt et al. (2014) and Perrone et al. (2017) also recommend that unusual fungi from metabolic uniqueness based on (a) the presence of an unreported pathway, (b) modification of an existing pathway, and (c) metabolic differences compared to the type strain be accessed in one, or (better) two internationally-recognised culture collections to enable identity and purity checking. MUM at the address above would be willing to accept these unusual strains described in Rharmitt et al. (2016) for confirmation of identity and further analytical studies by UHPLC DAD. The *idh* PCR using the original primers in Paterson et al. (2000) to avoid suboptimal binding would be employed. Finally, suggesting a different metabolic pathway for PAT production in these fungi is premature.

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