Evaluation of the presence of *IDH* gene and patulin production of *Aspergillus* and *Penicillium* strains isolated from Hungarian apples

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INTRODUCTION

Mycotoxins are secondary metabolites produced by fungal species that can be harmful to humans and animals. Patulin (PAT) is one of the toxins that can be found in different food products but especially in apples and derivates. The most common moulds recognized as producers of this compound belong to the species of *Aspergillus clavatus* and *Penicillium expansum*. The key enzyme in the patulin biosynthesis pathway is the isoepoxydon dehydrogenase enzyme (idh), encoded by *IDH* gene. Understanding the factors that can influence the production of PAT is an important tool to develop methods for detoxification and/or inhibition of this compound in food products. This work aimed to detect the presence of the *IDH* gene in different strains of *Aspergillus* and *Penicillium* and to subsequently evaluate the actual production of PAT using thin-layer chromatography (TLC) by those strains that harbour this gene.

METHODOLOGY

***Aspergillus* spp.**

- *A. clavatus* Br1
- *A. parasiticus*
- *A. versicolor* 1943
- *A. versicolor* 584,65
- *A tamarii* F2
- *A. niger*
- *A.* F 2/3
- *A.* F 3

***Penicillium* spp.**

- *P. brevicompactum* JR-3
- *P. brevicompactum* C'-3b
- *P. brevicompactum* P-3
- *P. expansum* A
- *P. expansum* C-4/1
- *P. expansum* F'2B

- Malt extract broth
  - 150 rpm
  - 35 °C
  - 72 h

DNA extraction
- MasterPure™ Yeast DNA Purification Kit

PCR amplification *IDH* gene
Primers
5`-CAA TGT GTC GTA CTG TGC CC-3`,
5`-ACCTTC AGT CGC TGT TCC TC-3`.
600 bp (Paterson et al., 2003)

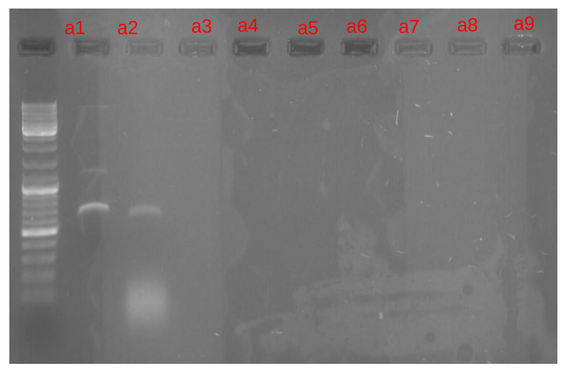
Electrophoresis
- 1% agarose

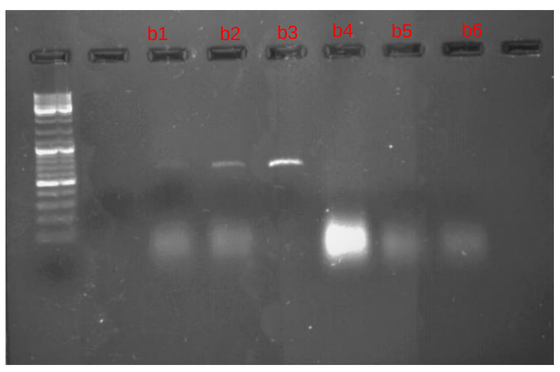
If positive for *IDH* gene

- Malt extract broth
  - 150 rpm
  - 35 °C
  - 7 days

Patulin Extraction
- Ethyl-acetate (2x)
- Evaporation
- Resuspension acetonitrile

TLC
- Mobile phase: toluene: ethyl acetate: formic acid (6:3:1)
- Revealing agent: 3-methyl-2-benzothiazolinone hydrazone





IDH gene PCR results for

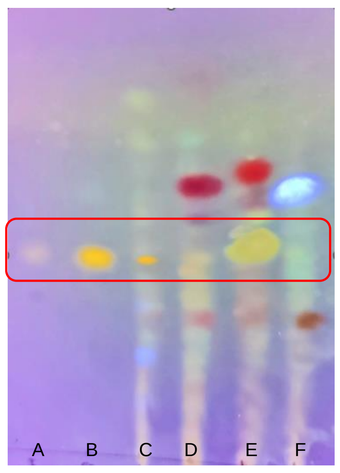
Aspergillus spp.

a1 and **a2 -** *A. clavatus* Br1*;*
a3-*A. parasiticus;*
a4-*A.versicolor* 1943*;*
a5- *A. versicolor* 584,65;
a6-*A tamarii* F2*;*
a7- *A. niger;*
a8-*A.* F2/3*;*
a9-*A.* F3

Penicillium spp.

b1-*P. expansum* A
b2-*P. brevicompactum P-3*
b3-*P. expansum F'2B*
b4-*P. expansum* C-4/1
b5- *P. brevicompactum* JR-3
b6-*P. brevicompactum C'-3b*

- *A. clavatus* Br1, *P. expansum* A, *P. expansum* F'2B *and P. brevicompactum* P-3, presented a bright mark around 600 pb indicating the presence of the *IDH* gene.



TLC results
A – Patulin standard 5ppm,
B - Patulin standard 50 ppm,
C - *A. clavatus* Br1,
D – *P. expansum* A,
E – *P. expansum* F'2B
F - *P. brevicompactum* P-3

- On the TLC plate patulin presents an intense yellow color under UV-light.
- The intensity and brightness can vary according to the concentration.
- For *A. clavatus* Br1 and *P. expansum* F'2B the mark for patulin is more intense.
- *P. expansum* A presents a faded yellow mark indicating a lower concentration of patulin.
- *For P. brevicompactum* P-3 no patulin production could be detected by this method.

CONCLUSIONS

- The strains *A. clavatus* Br1*, P. expansum* A*, P. expansum* F'2B and *P. brevicompactum P-3* presented positive results for the *IDH* gene.
- The strains *A. clavatus* B1, *P. expansum* A and *P. expansum* F'2B were able to produce patulin under the studied conditions.
- *P. brevicompactum P-3,* although presenting the *IDH* gene, was not able to produce patulin. This can be due to either the given environmental conditions affecting the gene regulation negatively or absence of other genes responsible for coding different steps for patulin production.

REFERENCE

Paterson, R.R.M., Kozakiewicz, Z., Locke, T., Brayford, D., Jones, S.C.B., 2003. Novel use of the isoepoxydon dehydrogenase gene probe of the patulin metabolic pathway and chromatography to test penicillia isolated from apple production systems for the potential to contaminate apple juice with patulin. Food Microbiology, 20(3), 359–364.

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