



Original

# The isoeoxydon dehydrogenase gene PCR profile is useful in fungal taxonomy

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**Summary** This study evaluates the specificity of PCR isoeoxydon dehydrogenase (*idh*) primers on fungi associated with patulin production. The DNAs of 93 strains were extracted and analysed by PCR using primers of the *idh* gene of patulin biosynthesis. A single band at 620 bp was obtained on 17% of the analysed strains. Different molecular weight amplicons were observed in other strains. These were employed as binary characters for numerical analysis to obtain a dendrogram. Clusters were observed, which corresponded to morphological identifications in some cases. Amplicons at 400 and/or 500 bp were related to patulin non-detection for strains, whereas a 450 bp amplicon was associated with some *Aspergillus* and both of the *Byssoschlamys nivea* strains tested. Hence, the *idh* primers are not specific for the gene and provide other amplicon products in other species. These results were useful providing (a) profiles of DNA to identify and classify fungi and (b) insights into patulin production. The DNA profiles in this study may be useful for determining patulin producing fungi. Obtaining multiple bands in culture-independent PCR of environmental samples by using the primers could indicate that more than one species is present.

**Key words** Isoeoxydon dehydrogenase gene, Patulin, PCR, Numerical analysis, *Penicillium*, *Aspergillus*, *Byssoschlamys*

## Utilidad en taxonomía fúngica de los patrones obtenidos por PCR del gen de la isoeoxydon deshidrogenasa

**Resumen** El interés del presente estudio fue investigar la especificidad de los cebadores para la PCR del gen de la isoeoxydon deshidrogenasa en hongos asociados a la producción de patulina. El DNA de 93 cepas fue extraído y analizado mediante PCR utilizando cebadores del gen *idh* implicado en la biosíntesis de la patulina. Se obtuvo una banda simple de 620 pb en un 17% de las cepas y bandas de peso molecular variable para el resto. Estos datos fueron utilizados como caracteres binarios en un análisis numérico. Se pudo comprobar que los diferentes clusters que aparecían en el correspondiente dendrograma a veces se correspondían con determinados caracteres morfológicos de las cepas. Amplificados de 400 y/o 500 pb se relacionaron con cepas no productoras de patulina y una banda de 450 pb se asoció con algunos *Aspergillus* y con las dos cepas de *Byssoschlamys nivea* incluidas en el estudio. Ello demostraba que los cebadores no eran específicos para el gen y amplificaban otras regiones en algunas especies. Estos resultados demostraron ser útiles en para la identificación y clasificación de hongos y para un mejor conocimiento de la producción de patulina. Determinados patrones de DNA pueden ser útiles para detectar hongos potenciales productores de patulina. La obtención de múltiples bandas en una PCR de muestras ambientales no cultivadas puede indicar que más de una especie está presente.

**Palabras clave** Gen de la isoeoxydon deshidrogenasa, Patulina, PCR, Análisis numérico, *Penicillium*, *Aspergillus*, *Byssoschlamys*

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Aceptado para publicación el 18 de julio de 2007

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Apdo. 699, E-48080 Bilbao (Spain)  
1130-1406/01/10.00 €

## Introduction

The significance of the polyketide patulin as a mycotoxin has increased greatly from the introduction of new European Union regulations that decrease the acceptable limits in fruit products and especially those used in baby foods. Evidence of this fact was recently reported in two patulin reviews [6,7]. Furthermore, the possibility for controlling the mycotoxin in food commodities has improved with greater understanding of the patulin metabolic pathway [17] and was further enhanced when the sequences of crucial genes were determined [4]. In particular, the sequencing of the isoeopoxydon dehydrogenase gene (*idh*) which produces the enzyme to transform isoeopoxydon to phyllostine, has been of greatest use.

Paterson et al. [8] were the first to employ *idh* primers to screen fungi specifically in the context of patulin as a mycotoxin: the fungi that were analysed were all penicillia. In addition, primers were employed seminally to analyse environmental samples as a cultural independent PCR (CIP). The analyses were extended to many other species within penicillia using culture dependant PCR (CDP), indicating that strains could be classified as to whether they were positive for *idh* and patulin detection [10]. Varga et al. [18] indicated the presence of *idh* and patulin production within *Aspergillus* section *Clavati* and included an evolutionary perspective on fungi with, or without, the trait. Paterson [12] analysed species from *Penicillium*, *Aspergillus*, and *Byssoschlamys* that were associated with patulin production: *B. nivea* was positive for *idh* and patulin detection. In addition, positive *idh* results were obtained for *Paecilomyces lilacinus* and a strain of *B. fulva* [11]. *Paecilomyces* is the anamorphic form of *Byssoschlamys*. Finally, an applied scheme for the classification of penicillia was developed using *idh* and patulin production as characters [13] to compliment other schemes e.g. Samson and Frisvad [16].

DNA sequencing demonstrated that the *idh* of *P. griseofulvum* was different from *P. expansum* [2] and that maximum parsimony trees based on rDNA and *idh* sequences were congruent [3] in the cases of these two important terverticillate penicillia. Unrelated *B. nivea* had a high degree of homology (88%) with the two penicillia: The *B. nivea* strains were identical independent of geographical region of isolation [1]. Puel et al. [15] determined that *B. fulva* could not produce patulin because of the absence of the 6-methylsalicylic acid synthase gene and *idh*. However, one other strain was *idh* positive in Paterson [11] and the situation requires clarification. White et al. [19] determined that *idh* was up-regulated under patulin permissive culture conditions.

During the investigations involving the use of *idh* primers on fungi it was noticed by the present author that other amplicons, apart from the expected product, appeared on gels at 620 bp. An assessment is made in the current report of the complete profiles for the classification of various fungi associated with patulin production using *idh* primers as such novel characters may be useful for the classification and identification of these organisms.

## Material and methods

Ninety-three strains were obtained from the CABI Bioscience, UK culture collection and were identified by expert taxonomists as associated with that organisation. Methods of growth were as in Paterson et al. [10]. Isolates were grown on slopes of potato dextrose agar or 2% (w/v) malt agar. The isolates were harvested and extracted. The

PCR analysis used has been described thoroughly [10] where images of representative gels are available. The band patterns were recorded to the nearest 50bp and subjected to numerical analysis using the statistical programme, Statistical Package for the Social Sciences version 14.0 and a dendrogram was obtained (Figure 1). However, the *idh* product was recorded as 620bp, which is the typical size. Control taxa were a *Ganoderma* sp. (IMI 357185), a basidiomycete that may cause disease in oil palm; and *Aspergillus flavus* (IMI 380661), which produces aflatoxins that are polyketides, as is patulin. Neither of these fungi is known to produce patulin.

The PCR mixture consisted of 200 mM of dNTPs (Pharmacia, Herts), 1.25 units of Tth polymerase, Tth buffer (both from HT Biotechnology, Cambridge, UK), 0.8

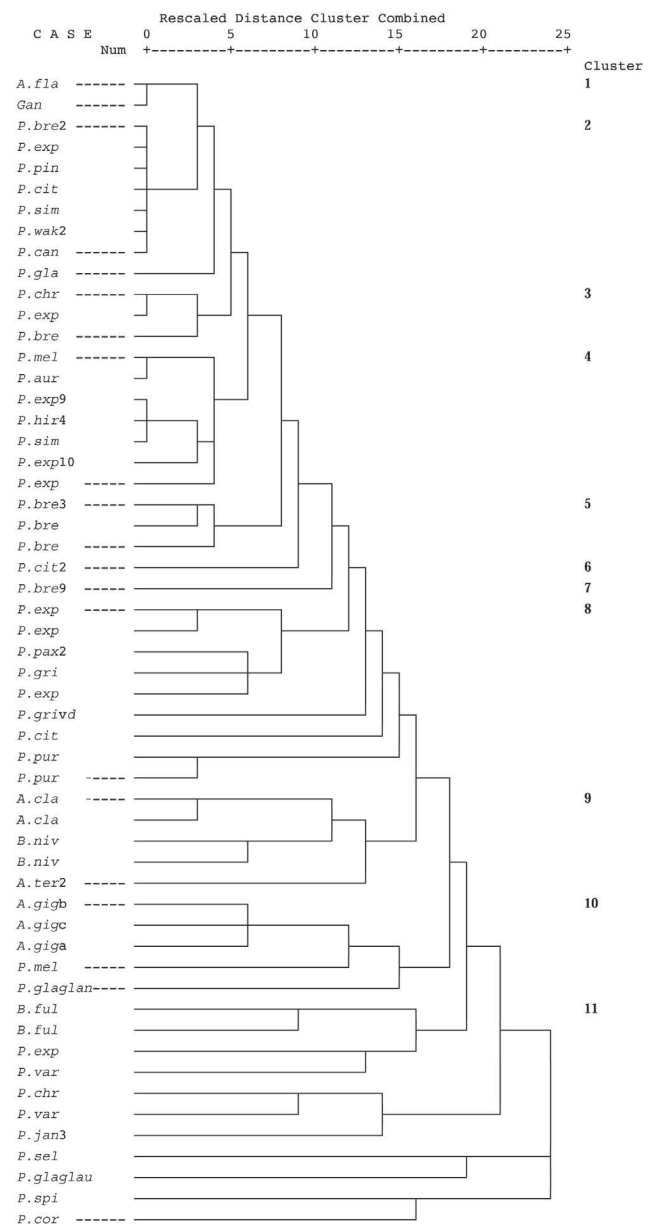


Figure 1. Dendrogram from a hierarchical cluster analysis by average linkage (between groups) of the bands resulting from the isoeopoxydon dehydrogenase primers applied to total DNA from the fungal strains. The numbers after the abbreviated species names are the number of strains of the same species that were analysed. The broken horizontal lines indicate the limits of each cluster. The full names of the species can be determined from table 2.

**Table 1.** List of strains employed and PCR products from the isoeopoxydon dehydrogenase gene primers. The six-figure number is the IMI culture collection identification code.

| Fungus                        | Species/strain   | PCR products (base pairs). <i>idh</i> is 620 |     |     |     |          |                            |
|-------------------------------|--|--|-----|-----|-----|----------|----------------------------|
| <i>Aspergillus</i>            |  |  |     |     |     |          |                            |
| Subgen. <i>Aspergillus</i>    |  |  |     |     |     |          |                            |
| Sect. <i>Flavi</i>            | <i>A. flavus</i> 380661  |  |     |     |     |          | 800                        |
| Sect. <i>Terrei</i>           | <i>A. terreus</i> 135817, 016043   | 250  | 450 |     | 620 |          | 1100                       |
| Subgen. <i>Fumigati</i>       |  |  |     |     |     |          |                            |
| Sect. <i>Clavati</i>          | <i>A. clavatus</i> 015949v   | 300  | 450 |     | 620 |          | 800 1200                   |
|                               | 232883   | 300  | 450 |     | 620 |          | 800 1100, 1200             |
|                               | <i>A. gigantus</i> 343711  | 350  |     | 500 | 620 | 700, 750 | 850 1100                   |
|                               | 016154   | 350  |     | 500 | 620 | 750      | 850                        |
|                               | 358435   | 350  |     | 500 | 620 |          | 850 1100                   |
| <i>Byssochlamys nivea</i>     | 361545   |  | 450 |     | 620 |          | 800 900 1050               |
|                               | 058423   |  | 450 |     | 620 |          | 800                        |
| <i>B. fulva</i>               | 058422   |  | 400 |     | 620 | 750      | 850 1050, 1100             |
|                               | 040021   | 200  | 400 | 500 | 620 | 750      | 850 1100, 1050             |
| <i>Ganoderma</i> sp.          | 357185   |  |     |     |     |          | 800                        |
| <i>Paecilomyces variotii</i>  |  |  |     |     |     |          |                            |
|                               | 321342   |  | 400 | 500 | 620 |          | 1050, 1300                 |
|                               | 204127   | 200  | 400 |     |     | 700      | 800 1050, 1300             |
| <i>Penicillium</i>            |  |  |     |     |     |          |                            |
| Subgen. <i>Aspergilloides</i> |  |  |     |     |     |          |                            |
|                               | <i>P. glabrum</i> 380331   |  |     |     |     |          | 1000                       |
|                               | <i>P. spinulosum</i> 380642  | 350  |     |     | 620 | 700      | 950 1050, 1200, 1500, 2000 |
| Subgen. <i>Biverticillium</i> |  |  |     |     |     |          |                            |
|                               | <i>P. pinophilum</i> 380659  | No bands                                     |     |     |     |          |                            |
|                               | <i>P. purpurogenum</i> 380952  |  |     | 500 |     | 700      | 1100, 1400                 |
|                               | 380948   |  |     | 500 |     |          | 1100, 1400                 |
|                               | <i>P. waksmanii</i> 381974, 381990   |  | 400 |     | 650 | 750      | 1200                       |
| Subgen. <i>Furcatum</i>       |  |  |     |     |     |          |                            |
|                               | <i>P. canescens</i> 380320   | No bands                                     |     |     |     |          |                            |
|                               | <i>P. citrinum</i> 380961, 380969  |  |     |     |     |          | 1250, 1400                 |
|                               | 380323   |  |     | 500 |     |          | 2000                       |
|                               | 380342   | No bands                                     |     |     |     |          |                            |
|                               | <i>P. corylophilum</i> 380322  | 350  |     |     | 620 |          | 1500, 1550, 2000           |
|                               | <i>P. janczewskii</i> 381948, 380968, 380633                                   |  |     |     | 550 |          | 800 900 1050, 1300         |
|                               | <i>P. melinii</i> 304279   | 350  | 400 |     | 620 |          | 850                        |
|                               | 040216ii   |  |     |     | 620 | 700      |                            |
|                               | <i>P. paxilli</i> 380639, 381272   |  | 400 |     |     | 750      |                            |
|                               | <i>P. selandiae</i> 304284   |  | 400 | 500 | 620 | 700      | 950 1000, 1400             |
|                               | <i>P. simplicissimum</i> 380333  |  |     |     | 620 |          |                            |
|                               | 380971   | No bands                                     |     |     |     |          |                            |
| Subgen. <i>Penicillium</i>    |  |  |     |     |     |          |                            |
|                               | <i>P. aurantiogriseum</i> 265302   |  |     |     | 620 | 700      |                            |
|                               | <i>P. brevicompactum</i>   |  |     |     |     |          |                            |
|                               | 381970, 380944, 380346, 380347, 380348, 380329, 380341, 380656, 380321         |  |     |     | 620 |          | 1150, 1050                 |
|                               | 380334   |  |     |     | 620 |          | 1500                       |
|                               | 380330   |  |     |     | 620 |          |                            |
|                               | 380352   |  |     |     |     |          | 900 1100                   |
|                               | 380349, 380643, 380648   |  |     |     |     |          | 900                        |
|                               | 380353   |  | 400 |     |     |          | 900                        |
|                               | 380634, 380645   | No bands                                     |     |     |     |          |                            |
|                               | <i>P. chrysogenum</i> 380958   |  |     |     |     |          | 1500                       |
|                               | 380332   |  |     |     |     | 700      | 1050, 1300                 |
|                               | <i>P. expansum</i>   |  |     |     | 620 |          |                            |
|                               | 381952, 381969, 381268, 380345, 380350, 380665, 380657, 380662, 380653         |  |     |     | 620 | 750      |                            |
|                               | 299046   |  |     |     | 620 |          |                            |
|                               | 380324, 380326, 380327, 380325, 380344, 380337, 380336, 380335, 381265, 380970 |  |     |     | 620 |          | 1200                       |
|                               | 232297   | 400  |     | 500 | 620 | 750      |                            |
|                               | 380960   | 400  |     |     | 620 | 750      | 1200                       |
|                               | 380654   |  |     |     | 620 | 750      | 1200                       |
|                               | 381266   |  |     |     |     |          | 1500                       |
|                               | 380636   | No bands                                     |     |     |     |          |                            |
|                               | 028619   | 200  | 400 | 500 | 620 |          | 1200                       |
|                               | <i>P. griseofulvum</i> 075832ii  | 300  | 400 |     | 620 | 750      |                            |
|                               | <i>P. griseofulvum</i>   |  |     |     |     |          |                            |
|                               | var. <i>dipodomycicola</i> 296935  | 350  |     |     | 620 | 750      | 900                        |
|                               | <i>P. glandicoladicola</i>   |  |     |     |     |          |                            |
|                               | var. <i>glandicoladicola</i> 321513  | 300  |     | 500 | 620 |          | 850 1150                   |
|                               | <i>P. glandicoladicola</i>   |  |     |     |     |          |                            |
|                               | var. <i>glaucovenetum</i> 321511   | 200, 300                                     | 400 | 500 | 620 | 700      | 900                        |
|                               | <i>P. hirsutum</i> 381967, 381951, 382063, 382064                              |  |     |     | 620 |          |                            |

mM of primer IDH1, and IDH2, and template DNA. The IDH gene was amplified at 94 °C for 1 min, 52 °C for 1 min, 72 °C for 1 min for 30 cycles with a Hybaid, Omn-E thermal cyclor. The initial denaturing step was 94 °C for 3 min and the programme was completed with an extension step of 72 °C for 5 min. The primers (GIBO BRL, Paisley) were: IDH1 sequence: 5k-CAATGTGTCGTACT GTGCC-3k, and IDH2 sequence: 5k-ACCTTCAGTCGCTGTTCCCTC-3k. A list of the 93 strains used is provided (Table 1) together with the DNA products observed.

## Results

A single amplicon at 620 bp was obtained by 17% of the 93 strains tested which were mainly strains of *P. simplicissimum*, *P. expansum*, *P. brevicompactum* and *P. hirsutum* (Table 1). The remainder strains showed 2 to 7 amplicons, with the exception of *P. spinulosum*, which showed 8 bands. The different banding patterns are shown in table 1 some of which are specific for species as revealed in the dendrogram (Figure 1). The strains representing each cluster are provided in table 2. From "top" to "bottom" of the dendrogram, the first cluster is formed by the *A. flavus* and the *Ganoderma* sp strains, (outgroup). The second cluster was made of strains, which bands were not apparent (Table 1). A single *P. glabrum* strain was loosely linked to this cluster. A cluster of three unrelated subgenus penicillia formed the fourth cluster. This was followed by a cluster containing most of the *P. expansum* strains and which were all *idh* positive. Approximately 50% of these strains produced also a band above 1000 bp. However, this expansum cluster included the *P. hirsutum* strains together with one *P. aurantiogriseum*. *P. melinii* and *P. simplicissimum* from subgenus Furcatum were also included in this cluster. A cluster of five *idh* negative *P. brevicompactum* strains was followed by a cluster of two *P. citrinum*. However, these were followed by most of the *P. brevicompactum* strains, which possessed the *idh* band at 620 bp and other higher molecular weight products at 1050 bp and 1150 bp. Those strains without the 620 amplicon showed a high molecular weight band at approximately 900 bp. A mixed collection of predominately single strains clustered below these strains, although the *P. paxilli* strains were identical and the *P. purpurogenum* were similar to each other in terms of the banding patterns. The next group was formed by *A. clavatus*, *A. terreus* and *B. nivea* strains, all of which were *idh* positive. However, *A. giganteus* clustered separately and were linked loosely to two penicillia. Strains with complex patterns containing an average of 5 bands were separate from the other strains and formed numerous "single strain clusters" towards the lower portion of the dendrogram. However, two strains of *B. fulva* clustered here and were *idh* positive: Three strains of *P. janczewskii* were *idh* negative and formed a loose group with two other *idh* negative strains of *Paecilomyces variotii* and *P. chrysogenum*. The other strains in this group were *idh* positive, the predominant bands being at 400 bp and 500 bp.

## Discussion

It is apparent that the *idh* primers were not specific for the expected 620 bp product. The bands produced did not allow for complete separation of taxa into individual clusters in all circumstances. Nevertheless, the banding patterns provided are useful taxonomic characters in addition to determining the presence of *idh*. A group of predominately *P. expansum* strains was observed, all of which

**Table 2.** Strains included in each cluster. The six-figure number is the IMI culture collection identification code.

| Clusters      | Fungi   |
|---------------|---|
| 1.            | <i>A. flavus</i> 380661, <i>Ganoderma</i> sp. 357185.   |
| 2.            | <i>P. pinophilum</i> 380659, <i>P. waksmanii</i> 381974, 381990.<br><i>P. citrinum</i> 380342, <i>P. simplicissimum</i> 380971,<br><i>P. canescens</i> 380320, <i>P. brevicompactum</i> 380634,<br>380645, <i>P. expansum</i> 380636.   |
| Single strain | <i>P. glabrum</i> 380331.   |
| 3.            | <i>P. brevicompactum</i> 380334, <i>P. chrysogenum</i> 380958,<br><i>P. expansum</i> 381266.  |
| 4.            | <i>P. expansum</i> 299046, 381952, 381969, 381268,<br>380345, 380350, 380665, 380657, 380662, 380653,<br>380324, 380326, 380327, 380325, 380344, 380337,<br>380336, 380335, 381265, 380970. <i>P. hirsutum</i> 381967,<br>381951, 382063, 382064. <i>P. aurantiogriseum</i> 265302.<br><i>P. melinii</i> 040216ii, <i>P. simplicissimum</i> 380333.                   |
| 5.            | <i>P. brevicompactum</i> 380352, 380349, 380643, 380648,<br>380353.   |
| 6.            | <i>P. citrinum</i> 380961, 380969.  |
| 7.            | <i>P. brevicompactum</i> 381970, 380944, 380346, 380347,<br>380348, 380329, 380341, 380656, 380321.   |
| 8.            | <i>P. expansum</i> 380960, 380654.<br><i>P. paxilli</i> 380639, 381272.<br><i>P. griseofulvum</i> 075832ii.<br><i>P. expansum</i> 232297<br><i>P. griseofulvum</i> var. <i>dipodomycicola</i> 296935<br><i>P. citrinum</i> 380323<br><i>P. purpurogenum</i> 380952, 380948.   |
| 9.            | <i>A. clavatus</i> 015949v, 232883.<br><i>B. nivea</i> 361545, 058423.<br><i>A. terreus</i> 135817, 016043.   |
| 10.           | <i>A. giganteus</i> 343711, 016154, 358435.<br><i>P. melinii</i> 304279<br><i>P. glandicoladicola</i> v. <i>glandicoladicola</i> 321513   |
| 11.           | <i>B. fulva</i> 058422, 040021.<br><i>P. expansum</i> 028619<br><i>Pa. variotii</i> 321342<br><i>P. chrysogenum</i> 380958<br><i>Pa. variotii</i> 204127<br><i>P. janczewskii</i> 381948, 380968, 380633<br><i>P. selandiae</i> 304284<br><i>P. glandicoladicola</i> var. <i>glaucovenetum</i> 321511<br><i>P. spinulosum</i> 380642<br><i>P. corylophilum</i> 380322 |

produced detectable amounts of patulin and were *idh* positive. Some non-*P. expansum* species were observed in the same cluster. However, these tended to be negative for patulin production as reported in [8,10,12]. Differences within *P. expansum* require further investigation as the fungus is an important pathogen of economic food plants and a known mycotoxin producer.

The *P. brevicompactum* strains consist of at least two separate groups, one of which contains strains that were positive for *idh*. These may form the other members of the *Olsonii* series, as the morphological methods employed to identify the fungi would not necessarily separate the different species of the series (see [14]). The *Aspergillus* and *B. nivea* were separate from *Penicillium*, as would be anticipated from conventional taxonomic analysis. Related to this, the *A. clavatus*, *A. terreus* and *B. nivea* all provided a unique amplicon at 450 bp compared to the other species. More work is necessary to determine if this is a

genuine difference. The *B. nivea* were also separate from *B. fulva*, which is interesting as these species are difficult to differentiate by conventional methods. It is notable that the *B. fulva* strains were positive for *idh*, contradicting the hypothesis of Puel et al. [15] that *B. fulva* are not capable of producing patulin because the species does not possess *idh*, amongst other genes. It is necessary to consider the possibility of inhibition of PCR in the circumstances where CDP is employed [5,9]. The other banding patterns obtained provide additional characters for identification of taxa.

In conclusion, PCR using *idh* primers provides more information than a simple, single band at 620 bp. This needs to be appreciated when undertaking CIP analysis where many bands can be obtained [8] and, for example, multiple bands may not be indicative that numerous

species could be present in environmental samples. The data provided additional taxonomic characters that may be useful in classification and identification of species, in addition to indicating whether strains possess the *idh* gene.

*RRM Paterson is funded by grant SFRH/BPD/34879/2007 from Fundação para a Ciência e a Tecnologia, Portugal. Dr Zofia Kozakiewicz is acknowledged gratefully for the morphological identifications which she undertook.*

## References

1. Dombrink-Kurtzman MA, Engberg AE. *Byssoschlamys nivea* with patulin-producing capability has an isoeopoxydon dehydrogenase gene (*idh*) with sequence homology to *Penicillium expansum* and *P. griseofulvum*. *Mycol Res* 2006; 110: 1111-1118.
2. Dombrink-Kurtzman MA. The isoeopoxydon dehydrogenase gene of the patulin metabolic pathway differs for *Penicillium griseofulvum* and *Penicillium expansum*. *Anton van Leeuw* 2006; 89: 1-8.
3. Dombrink-Kurtzman MA. The sequence of the isoeopoxydon dehydrogenase gene of the patulin biosynthetic pathway in *Penicillium* species. *Anton van Leeuw* 2006; 91: 179-189.
4. Fedeshko RW. Polyketide enzymes and genes in *Penicillium urticae*. PhD Dissertation, University of Calgary, Alberta, Canada 1992.
5. Hoorfar J, Cook N, Malorny B, de Medici D, Abdulmawjood A, Fach P. Diagnostic PCR: making internal amplification control mandatory. *J Appl Microbiol* 2004; 96: 221-222.
6. Jackson L, Dombrink-Kurtzman MA. Patulin. In: Sapers GM, Gorny JR, Yousef AE (Eds.) *Microbiology of Fruits and Vegetables*. Boca Raton, FL, CRC Press, 2006: 281-311.
7. Moake MM, Padilla-Zakour OI, Worobo RW. Comprehensive Review of Patulin Control Methods in Foods. *Comp Rev Food Sci Food Safety* 2005; 1: 8-21.
8. Paterson RRM, Archer S, Kozakiewicz Z, Lea A, Locke T, O'Grady E. A gene probe for the patulin metabolic pathway with potential use in novel disease control. *Biocon Sci Technol* 2000; 10: 509-512.
9. Paterson RRM. Internal amplification controls have not been employed in diagnostic fungal PCR hence potential false negative results. *J Appl Microbiol* 2007; 102: 1-10.
10. Paterson RRM, Kozakiewicz Z, Locke T, Brayford D, Jones SCB. Novel use of the isoeopoxydon 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 Microbiol* 2003; 20: 359-364.
11. Paterson RRM. The isoeopoxydon dehydrogenase gene of patulin biosynthesis in cultures and secondary metabolites as candidate PCR inhibitors. *Mycol Res* 2004; 108: 1431-1437.
12. Paterson RRM. The isoeopoxydon dehydrogenase gene of the patulin biosynthetic pathway, patulin detection, and the utility of species names in patulin-producing fungi. In: Lima N, Smith D (Eds.) *Biological Resource Centres and the use of Microbes*, European Culture Collections Organisation XXII. Braga, Micoteca da Universidade do Minho, 2003; 259-266.
13. Paterson RRM, Venâncio A, Lima, N. A novel identification system based on 318 penicillia strains using the isoeopoxydon dehydrogenase gene and patulin production. *Rev Iberoam Micol* 2006; 23: 155-159.
14. Peterson SW. Multilocus DNA sequence analysis shows that *Penicillium biourgeianum* is a distinct species closely related to *P. brevicompactum* and *P. olsonii*. *Mycol Res* 2004; 108: 434-440.
15. Puel O, Tadriss S, Delaforge M, Oswald I, Lebrihi, A. The inability of *Byssoschlamys fulva* to produce patulin is related to absence of 6-methylsalicylic acid synthase and isoeopoxydon dehydrogenase genes. *Inter J Food Microbiol* 2006; 115: 131-139.
16. Samson RA, Frisvad JC. *Penicillium* subgenus *Penicillium*: New taxonomic schemes, mycotoxins and other extrolites. *Stud Mycol* 2004; 49: 1-258.
17. Sekiguchi J, Shimamoto T, Yamada Y, Gaucher GM. Patulin biosynthesis: enzymatic and nonenzymatic transformations of the mycotoxin (E)-ascladiol. *Appl Environ Microbiol* 1983; 45: 1939-1942.
18. Varga J, Rigó K, Molnár J, Tóth B, Szencz A, Téren J, Kozakiewicz Z. Mycotoxin production and evolutionary relationships among species of *Aspergillus* section *Clavati*. *Anton van Leeuw* 2003; 83: 191-200.
19. White S, O'Callaghan J, Dobson ADW. Cloning and molecular characterization of *Penicillium expansum* genes upregulated under conditions permissive for patulin biosynthesis. *FEMS Microbiol Lett* 2006; 255: 17-26.