Inter-laboratory trials for proficiency testing of mycological laboratories

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ABSTRACT

A project was started in Germany in 2001 to develop and establish inter-laboratory trials for proficiency testing of mycological laboratories analysing fungi in indoor environments.

Four inter-laboratory trials have been performed using pure cultures. Six strains of fungi relevant to indoor environments have been sent to the participating laboratories for genera and species identification during each trial. A total of 40–60 laboratories participated in each trial and 50–80% of them met the requirements for a successful participation. In a second part of the project, natural samples with mixed fungal species were sent to the participants. The results revealed difficulties in preparing and analysing such samples.

The inter-laboratory trials have increased the quality of the analysis in the participating laboratories. The statistical evaluation showed that laboratories that took part more than once in the trials were more successful than newcomers. It is planned to carry out the inter-laboratory trials at the international level.

INDEX TERMS

Inter-laboratory trials; Proficiency testing; AQC; Fungi; Mould; Species differentiation

INTRODUCTION

Differentiation of fungi to genera or species level is considered very important in detecting sources of fungal growth in indoor environments and for assessing the allergenic, pathogenic or immunotoxigenic potential of the fungi detected.

The German Federal Environmental Agency has published a guide for the prevention, investigation, evaluation and remediation of fungal growth in indoor environments. The recommendations for detection and assessment of fungal growth are based on differentiation of fungi to the genera or species level. Since this requires a high degree of competence of the analytical laboratories, the German Federal Environmental Agency has appointed the Health Agency of the State of Baden-Württemberg to establish an inter-laboratory trial for proficiency testing of mycological laboratories.

RESULTS

Four inter-laboratory trials for the identification of indoor fungi have been performed so far. For each trial, six strains of fungi relevant to indoor environments were sent to the participating laboratories. Participants were provided with a list of relevant fungi which might be included in the trials (see Table 1).

The cultures were checked for purity and expression of typical morphological characteristics by eight reference laboratories prior to the trial. The prerequisite to use a strain in the inter-laboratory trials was that all reference laboratories had correctly identified the species. Internal quality control measures in the coordinating laboratory to guarantee the

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purity and identity of the strains have proven to be very important before dispatching the cultures.

Table 1 List of fungi relevant to indoor environments that might be included in the inter-laboratory trials

Absidia corymbifera	*Gliocladium roseum*
Acremonium kiliense	Yeasts
Acremonium murorum	*Memnoniella echinata*
Acremonium strictum	*Mucor hiemalis*
Alternaria alternata	*Mucor plumbeus*
Aspergillus fumigatus	*Mucor racemosus*
Aspergillus niger	*Oidiodendron griseum*
Aspergillus ochraceus	*Paecilomyces variotii*
Aspergillus penicillioides	*Penicillium aurantiogriseum*
Aspergillus restrictus	*Penicillium brevicompactum*
Aspergillus sydowii	*Penicillium chrysogenum*
Aspergillus tamarii	*Penicillium citrinum*
Aspergillus terreus	*Penicillium digitatum*
Aspergillus ustus	*Penicillium commune*
Aspergillus versicolor	*Penicillium corylophilum*
Aspergillus wentii	*Penicillium expansum*
Aureobasidium pullulans	*Penicillium glabrum*
Beauveria bassiana	*Penicillium funiculosum*
Botrytis cinerea	*Penicillium griseofulvum*
Byssochlamys nivea	*Penicillium purpurogenum*
Chaetomium globosum	*Penicillium olsonii*
Chrysonilia crassa	*Penicillium roquefortii*
Chrysonilia sitophila	*Penicillium variabile*
Chrysosporium sp.	*Phialophora fastigiata*
Cladosporium cladosporioides	*Phoma glomerata*
Cladosporium herbarum	*Phoma macrostoma*
Cladosporium sphaerospermum	*Rhizopus stolonifer*
Curvularia geniculata	*Rhodotorula minuta*
Doratomyces sp.	*Scopulariopsis brevicaulis*
Emericella nidulans	*Scopulariopsis fusca*

Engyodontium album	*Stachybotrys chartarum*
Epicoccum nigrum	*Stemphylium botryosum*
Eurotium amstelodami	*Syncephalastrum racemosum*
Eurotium chevalieri	*Trichoderma harzianum*
Eurotium herbariorum	*Trichoderma viride*
Eurotium rubrum	*Trichothecium roseum*
Fusarium culmorum	*Ulocladium chartarum*
Fusarium oxysporum	*Verticillium lecanii*
Fusarium solani	*Verticillium luteoalbum*
Geomyces pannorum	*Wallemia sebi*

The participating laboratories were given 6 weeks for identification of the test strains. A successful participation was only confirmed if the participants had identified correctly four out of six strains up to the species level. In the first three trials, about 40 laboratories participated and 60–80% met the requirements. In the fourth trial 64 labs took part and half of them met the requirements. In total, 109 different laboratories participated, predominantly from Germany, but also from Austria, Belgium, Portugal, Sweden, Switzerland and The Netherlands.

Problems were mainly encountered with the identification of *Penicillium* species. *Penicillium digitatum* was, for example, correctly identified by only one laboratory in the first trial and by nine laboratories in the third trial. Several laboratories had, in addition, problems in correctly identifying the *Aspergillus* species (see Table 2).

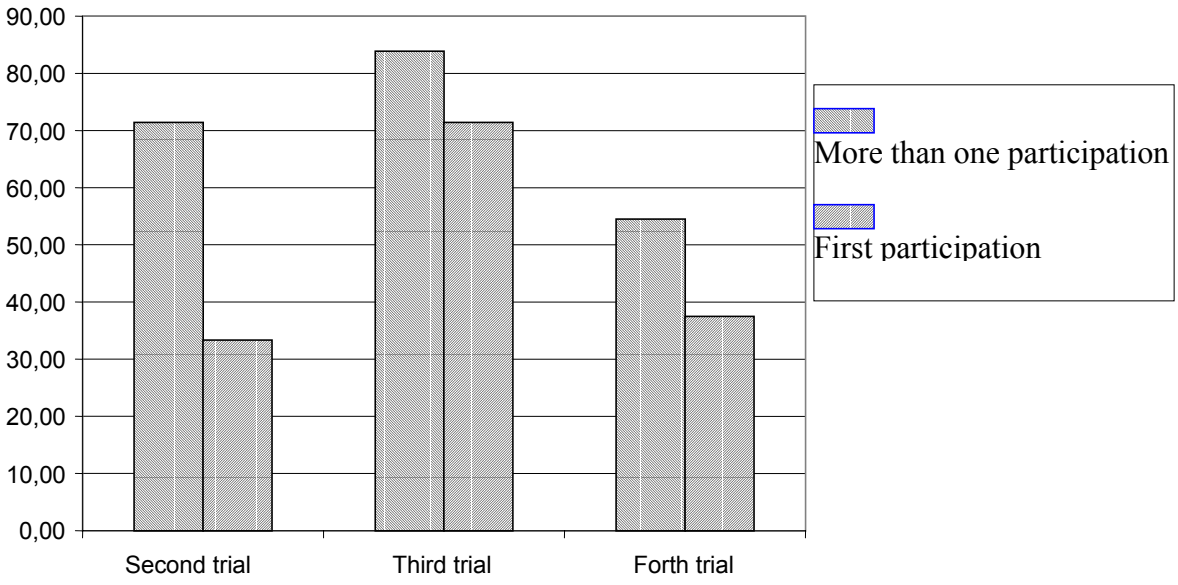
Table 2 Cultures used in the four inter-laboratory trials and percentage of laboratories which correctly identified the species

1. Trial	2. Trial	3. Trial	4. Trial
Aspergillus penicillioides (70)	*Aureobasidium pullulans* (87)	*Aspergillus fumigatus* (90)	*Aspergillus restrictus* (39)
Emericella nidulans (84)	*Penicillium digitatum* (20)	*Cladosporium cladosporioides* (79)	*Penicillium olsonii* (49)
Penicillium digitatum (7)	*Aspergillus versicolor* (58)	*Syncephalastrum racemosum* (90)	*Penicillium expansum* (49)
Rhizopus stolonifer (89)	*Mucor racemosus* (87)	*Chaetomium globosum* (83)	*Aspergillus candidus* (92)
Scopulariopsis brevicaulis (84)	*Penicillium chrysogenum* (67)	*Acremonium murorum* (50)	*Aspergillus ustus* (58)
Wallemia sebi (91)	*Eurotium amstelodami* (56)	*Stachybotrys chartarum* (83)	*Phoma glomerata* (68)

The inter-laboratory trials have, nevertheless, increased the quality of the analysis in the participating laboratories. The statistical evaluation showed that laboratories that took part more than once in the trials were more successful than newcomers (see Figure 1).

Natural samples with mixed fungal species were sent to the participants in a second part of the project. The results revealed difficulties in preparing and analysing such samples. More developmental work has to be done before natural samples can be used routinely for inter-laboratory trials.

Figure 1: Number of laboratories (in percent) which successfully completed the trial



CONCLUSIONS AND IMPLICATIONS

Differentiation of fungi to the genera and species level requires special expertise in the analysing laboratory. Practical exercises for continuous education are important to keep a high competence in the laboratories.

Inter-laboratory trials using pure cultures have revealed deficiencies in identifying fungal species especially of the genera *Penicillium* and *Aspergillus* in many laboratories. The quality of the analysis increased when laboratories regularly participated in inter-laboratory trials.

Inter-laboratory trials for proficiency testing should be introduced into the AQC schemes of all laboratories carrying out analysis of fungi in indoor air.

It is planned to carry out the inter-laboratory trials at the international level.

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