STUDIES IN MYCOLOGY 49: 243-251, 2004

Classification of Terverticillate *Penicillia* by Electrospray Mass Spectrometric Profiling

Jørn Smedsgaard*, Michael Edberg Hansen and Jens C. Frisvad

*Center for Microbial Biotechnology, BioCentrum-DTU, Technical University of Denmark, Søltofts Plads Build. 221, DK-2800 Kgs. Lyngby, Denmark. *corresponding author js@biocentrum.dtu.dk.*

Abstract: 429 isolates of 58 species belonging to *Penicillium* subgenus *Penicillium* are classified from direct infusion electrospray mass spectrometry (diMS) analysis of crude extracts by automated data processing. The study shows that about 70% of the species can be classified correctly into species using only the analysis of metabolites produced on one growth medium. This classification is in concurrence with the taxonomic delimitation of the accepted species obtained by a polyphasic approach. Other relations between species can be read from the dendrograms and the efficient classification shows the potential of this semi- automated identification system.

Keywords: electrospray mass spectrometry. taxonomy, subgenus *Penicillium*,

Introduction

Species belonging to *Penicillium* subgenus *Penicillium* are well known to produce a broad range of extrolites whereof many are known as mycotoxins (Frisvad & Samson 2004). Furthermore, the chemical structures of many metabolites have not been elucidated. Several studies have shown that the production of these extrolites form profiles which are unique to each species and therefore allow classification and identification of these species (Frisvad 1994).

The first use of extrolites for identification of fungi was the agar-plug TLC method by Filtenborg *et al*. (Filtenborg & Frisvad 1980; Filtenborg *et al.* 1983). This method is based on TLC profiles made directly from agar plugs cut from cultures that after selective spraying can be was to aid identification of the species. This method was later extended where extracts of cultures were analysed by HPLC (Frisvad 1987; Frisvad & Thrane 1987). Svendsen and Frisvad (1994) used extensive HPLC-UV analysis of species in the subgenus *Penicillium* to select diagnostic metabolites and grouped into chromophore families based on similarity of UV spectra that indicate a structural resemblance (Svendsen & Frisvad 1994). Their study showed that these species could be classified by their pattern of these selected metabolites as seen as peaks in HPLC-UV analyses using both the pattern of known or distinct unique unknown metabolites. Furthermore, they showed that the species delimitation agreed the classical phenotypical characterization The HPLC-UV method is rather laborious and time consuming as compared to the agar-plug TLC method, but it gives much more information about the unknown metabolites than TLC. The extraction procedure was much simplified by Smedsgaard (1997a) which combined with optimised HPLC analyses greatly increased the sample throughput.

A major limitation of the TLC and HPLC approaches is that they require experience to recognize the significant spots on a TLC plate or peaks/UV-spectra in a HPLC chromatogram. This can be assisted by analysis of metabolite standards and by the use of reference data (e.g. Frisvad & Thrane 1993; Nielsen & Smedsgaard 2003). However, the data evaluation is time consuming and difficult to automate (Nielsen *et al.* 1998; 1999).

In the beginning of the 1990's the commercialisation of electrospray mass spectrometry (ES-MS) greatly expanded the range of compounds that can be analysed by MS. A major feature of ES-MS is that the ionisation is very soft and by optimisation will produce mostly protonated or sodiated ions at low molecular mass. Therefore ES-MS allow a broad range of extrolites from fungi to be determined as their protonated or in some cases as their sodiated molecular ion with very high sensitivity (Smedsgaard & Frisvad, 1996). As ES-MS is compatible with HPLC, mass information can be obtained along with e.g. retention times and also UV spectra. These techniques are now in routine use in many laboratories and reference data have been published (e.g. Nielsen & Smedsgaard 2003). But as mentioned above processing of LC-MS data is also laborious, requires some expertise and is difficult to automate.

The most direct approach is to infuse the crude extracts directly into the electrospray source of the mass spectrometer (ES-MS) in positive mode to get a mass spectrum or rather a *mass profile* of the compounds in the sample, thus omitting the chromatographic column (Smedsgaard & Frisvad, 1996).

These profiles can be measured in the order of minutes and are easily adapted for automated processing, see below. Matrix effects can interfere significant in ES-MS spectra of complex mixtures, most notably by ion suppression where ions at high concentration, high surface potential or high proton affinity suppress other ions. However, it was demonstrated by Smedsgaard and Frisvad (1996) that the *Penicillium* species in Series *Viridicata* could be classified from their quite unique ES-MS spectra (mass profiles). These nominal mass spectra also showed ions corresponding to the protonated molecular mass of many of the metabolites produced by these species (Smedsgaard & Frisvad 1996). In a later study Smedsgaard & Frisvad (1997) included most species of subgenus *Penicillium* of which about 72% could be classified correctly (36 species out of about 50 species from 339 isolates examined on two media). Although a direct infusion mass spectrum cannot in its own right be used to identify metabolites, it can give an indication of the occurrence of a particular metabolite. The major advantage of direct infusion nominal mass spectra is that they are well suited for automated data processing and most mass spectrometers comes with software for library storage and retrieval. An efficient use of standard library software was demonstrated in an extension of the above study by Smedsgaard (1997b).

The most recent generation of mass spectrometers have the ability to produce high resolution spectra with a mass accuracy in the range of 5 ppm maintaining a very high sensitivity. By this accuracy only relatively few different formulae are possible for each ion seen in the spectrum, thus it is much more likely that different masses originate from different metabolites. However, reaching the high mass accuracy requires an internal mass reference. As at least one metabolite is known from most *Penicillium* species, these metabolites can be used as a native internal mass reference which can be used to correct the spectrum e.g. roquefortine C with the protonated mass 390.1930 Da is produced by about half of the terverticillate Penicillia. These accurate high resolution mass profiles contain much more information than nominal mass spectra, thus a better chemoclassification can be expected. Furthermore, due to the high precision, only a few structures are possible for each ion (in the range of 3-9 depending on mass and accuracy) giving a much more reliable confirmation of metabolite production.

Automated processing of high resolution mass spectra requires a somewhat different approach than nominal mass spectra. Whereas a series of nominal mass spectra easily can be transform into a grid structure (aligned) of integer mass without loss of information, this cannot be done as easily from accurate spectra. In an accurate spectrum the masses falls on a continuous mass scale, therefore it is not straight forward to find out which mass compares to which. The approach mostly used is binning, where a narrow grid is designed to fit over the data in such a way that each ion in each spectrum is placed in designated bins, e.g. using a bin width of 0.1 Da will combine ion with masses in the interval 252.0000 Da to 252.1000 Da into the same bin thus to be used as one variable for further data processing. Selection of bin width depends on mass resolution and accuracy. A more efficient method is the accurate mass spectrum (AMS) distance (Hansen & Smedsgaard 2004a; 2004b) by which the similarity between both accurate and nominal spectra can be calculated without alignment of the spectra (thus without applying a grid for binning the ions into variables). In short a correspondence map is determined by matching ion peaks pairs across two spectra according to the shortest distance. For each pair of corresponding masses, the difference is calculated giving a qualitative distance measure between "matched" masses. From this vector of differences the 90% quantile is calculated, defining the distance which at least 10% of the peaks have to be moved in order to give a perfect match. Finally, the correlation is calculated between the normalised intensities for all matched peak in each pair of spectra giving a correlation of one if there is a perfect match of peak heights. The product of the distance dissimilarity and (1 minus the intensity correlation) is collected into a dissimilarity matrix for all combinations of spectra.

The objective of this study was to test the classification of the 429 isolates representing 58 species belonging to subgenus *Penicillium* proposed by Frisvad and Samson (2004) with direct infusion ESMS accurate mass spectra.

Material and methods

Species from the study by Frisvad and Samson on the terverticillate Penicillia (Frisvad & Samson 2004), (58 species, approx. 8 isolates of most species, together 429 isolates) were analysed by direct infusion ES-MS. Each isolate was cultivated on CYA and YES agar (Samson *et al.* 2004) and incubated for 7 days in the dark at 25°C. Extracts were prepared from both the CYA and YES cultures by the plug extraction procedure (Smedsgaard 1997a) modified to use a two-step extraction procedure. Three 6 mm agar plugs were extracted using 0.5 ml ethyl acetate containing 0.5% formic acid in the first extraction and 0.5 ml 2-propanol in the second extraction. The combined extracts was evaporated to dryness and redissolved in methanol and filtered before injection into the mass spectrometer.

Table 1. Metabolites used as internal mass reference for mass scale correction. Prioritised, thus first mass reference 1 is searched in the spectrum, if not found then a search for mass reference 2 is performed. If neither masses are found no internal correction is performed. The metabolites are selected from Frisvad et al. (2004) on the basis of their general occurrence and good ionisation properties.

First choice		
Metabolite	**M+H$^+$**	**Species**
anacine	343.1770	*P. thymicola, P. nordicum*
asperphenamate	507.2284	*P. bialowiezense, P. olsonii*
aurantiamine	303.1821	*P. aurantiogriseum, P. neoechinulatum*
brevianamide A	311.1032	*P. brevicompactum*
cyclopenol	311.1032	*P. freii , P. discolor, P. echinulatum, P. solitum*
cyclopeptin	281.1290	*P. caseifulvum*
cyclopiazonic acid	337.1552	*P. palitans, P. dipodomyicola, P. commune, P. camemberti*
deoxybrevianamide E	352.2025	*P. italicum, P. ulaiense*
dipodazin	242.0929	*P. cavernicola, P. dipodomyis*
glyanthrypine	346.1555	*P. gladioli*
griseofulvin	353.0792	*P. aethiopicum*
nalgiovensin	329.1025	*P. nalgiovense*
norlichexanthone	259.0606	*P. clavigerum*
patulin	155.0344	*P. formosanum*
puberuline	444.2287	*P. cyclopium, P. polonicum, P. tricolor*
roquefortine C	390.1930	*P. chrysogenum, P. expansum, P. marinum, P. griseofulvum, P. paneum, P. albocoremium, P. allii, P. concentricum, P. confertum, P. coprobium, P. coprophilum, P. flavigenum, P. glandicola, P. radicicola, P. atramentosum, P. melanoconidium, P. crustosum, P. hirsutum, P. hordei, P. roqueforti, P. sclerotigenum, P. tulipae, P. venetum, P. vulpinum, P. carneum*
tryptoquialanine A	547.2192	*P. digitatum*
verrucin A	377.1613	*P. verrucosum*
verrucologen	400.2752	*P. mononematosum*
viridic acid	455.2294	*P. viridicatum*

Second choice		
alantrypinone	375.1457	*P. thymicola*
asperfuran	219.1021	*P. clavigerum*
asteltoxin	419.2070	*P. cavernicola*
auranthine	331.1195	*P. aurantiogriseum*
aurantiamine	303.1821	*P. freii*
brevianamide A	366.1817	*P. viridicatum*
chrysogine	191.0820	*P. chrysogenum*
communesin B	509.2916	*P. expansum, P. marinum*
cyclopaldic acid	239.0555	*P. mononematosum*
cyclopenol	311.1032	*P. neoechinulatum*
fumigaclavine A	299.1759	*P. palitans*
griseofulvin	353.0792	*P. dipodomyicola, P. griseofulvum*
marcfortin A	478.2706	*P. paneum*
meleagrin	434.1828	*P. albocoremium, P. allii, P. concentricum, P. confertum, P. coprobium, P. coprophilum, P. flavigenum, P. glandicola, P. radicicola*
mycophenolic acid	321.1338	*P. bialowiezense, P. brevicompactum, P. carneum*
ochratoxin A	404.0901	*P. nordicum, P. verrucosum*
oxaline	448.1985	*P. atramentosum, P. melanoconidium*
penicillin G	335.1065	*P. nalgiovense*
rugulovasine A	334.1555	*P. commune*
tryptoquialanine A	547.2192	*P. aethiopicum*
verrucofortine	410.2443	*P. cyclopium, P. polonicum, P. tricolor*

The sample was analysed by direct injection positive electrospray mass spectrometry (di-ESMS) on a Micromass Q-Tof time of flight mass spectrometer with a 3.6 GHz time-to-digital detection. The general procedure (Smedsgaard & Frisvad 1996) was modified to add the modifiers (formic acid and water) online by a syringe pump rather than adding modifier to the samples. 1 µl extract was infused at 15 µl/min by a methanol carrier flow. Just prior to the source water containing 2% formic acid was added at a rate of 5 µl/min giving a combined flow of 20 µl/min going into the source. Continuum scans was collected at a rate of 1 scan per second from m/z 150 to 1000 with 0.1 second interscan time. Data was collected from 0 to 2 minutes after injection and samples were injected at approx. 3 minutes interval. The instrument was tuned to a resolution better than 8500 and calibrated on a solution of PEG giving a residual error less than 2 mDa for all reference peaks (more than 28) by a 5^{th} order calibration.

Data processing

From each file a centroid mass spectrum were calculated by combining the scans during the elution of the extract, followed applying the calibration collected at the time of analysis and finally using one of the prioritised metabolites, see table 1, as internal mass references to correct the mass scale for optimum accuracy (Hansen & Smedsgaard 2004a; 2004b). These corrected centroid spectra were used for classification and collected in a chemotaxonomic database.

A classification was done using accurate mass spectrum (AMS) distance (Hansen & Smedsgaard 2004a) as described in the introduction. The data from cultures on YES and CYA are calculated separately. From the two dissimilarity matrices dendrograms (trees) are calculated by cluster analysis using UPGMA linkages. The raw data files were processed using in-house written software (see www.metabolomics.biocentrum.dtu.dk) and statistical analysis using the "R" statistical package (that can be downloaded from www.r-project.org) and NTSys 2.11 (Exeter Software, NY, USA).

Results and discussion

Figure 1 illustrates the structure of the raw data and the pre-processing. The elution profile at the top shows the crude extract eluting into the source. Scans are collected in the raw continuum format during the elution where data points are sampled at a fixed rate of about 139 points per Dalton (approx. 118000 point per spectrum). About 60 continuum scans are summarised into one continuum scan from which a centroid (stick) mass spectrum is calculated and the mass scale is corrected using an internal mass correction. This is done by search for ions corresponding to the metabolites and protonated masses given in table 1. If an ion is found the mass scale is corrected otherwise it used directly. In general a mass accuracy in the range 5 to 10 ppm can be obtained by internal mass correction, whereas the uncorrected accuracy is around 200 ppm. These centroid spectra are used for all further data processing. Between 100 and 600 ions are detected from each sample. Dendrograms from classifications by AMS dissimilarity - cluster analysis are shown on figure 2 and 3 keeping data from YES and CYA cultures separate. All in all 429 spectra were included in each analysis representing 57 species included. The overall performance is summarized in table 2.

Using a strict criterion, 26 species were classified correctly from the YES data and 22 from the CYA data according to the study by Frisvad and Samson (2004). There is consensus on 16 taxa by analyses from both media, further 10 are correctly classified by the YES data and another 6 species are correctly classified by the CYA data. The strict criterion requires that all isolates of a given species are grouped with other isolates of the same species as the closest neighbours.

Often a single isolate of a particular species shows poor growth, poor metabolite production or is missing a single characteristic metabolite, maybe due to long storage in a culture collection, therefore is too different from other isolates of the same species to end up in the correct cluster. If we accept clusters missing one isolate as correct classification, a further 13 species are clustered correctly from the YES data and 4 from the CYA data.

Table 2 Performance of the classification by cluster analysis from direct infusion ESI-MS analysis of crude extracts. Number of species classified correctly according to (Frisvad and Samson 2004) out of the 57 species examined.

	YES	CYA
Perfect classification of all examined isolates in unique cluster	26	22
Unique cluster missing one isolate	13	4
Perfect classification of all examined isolates in unique cluster but with alien isolate included	2	0
Considered correct	70 %	46 %

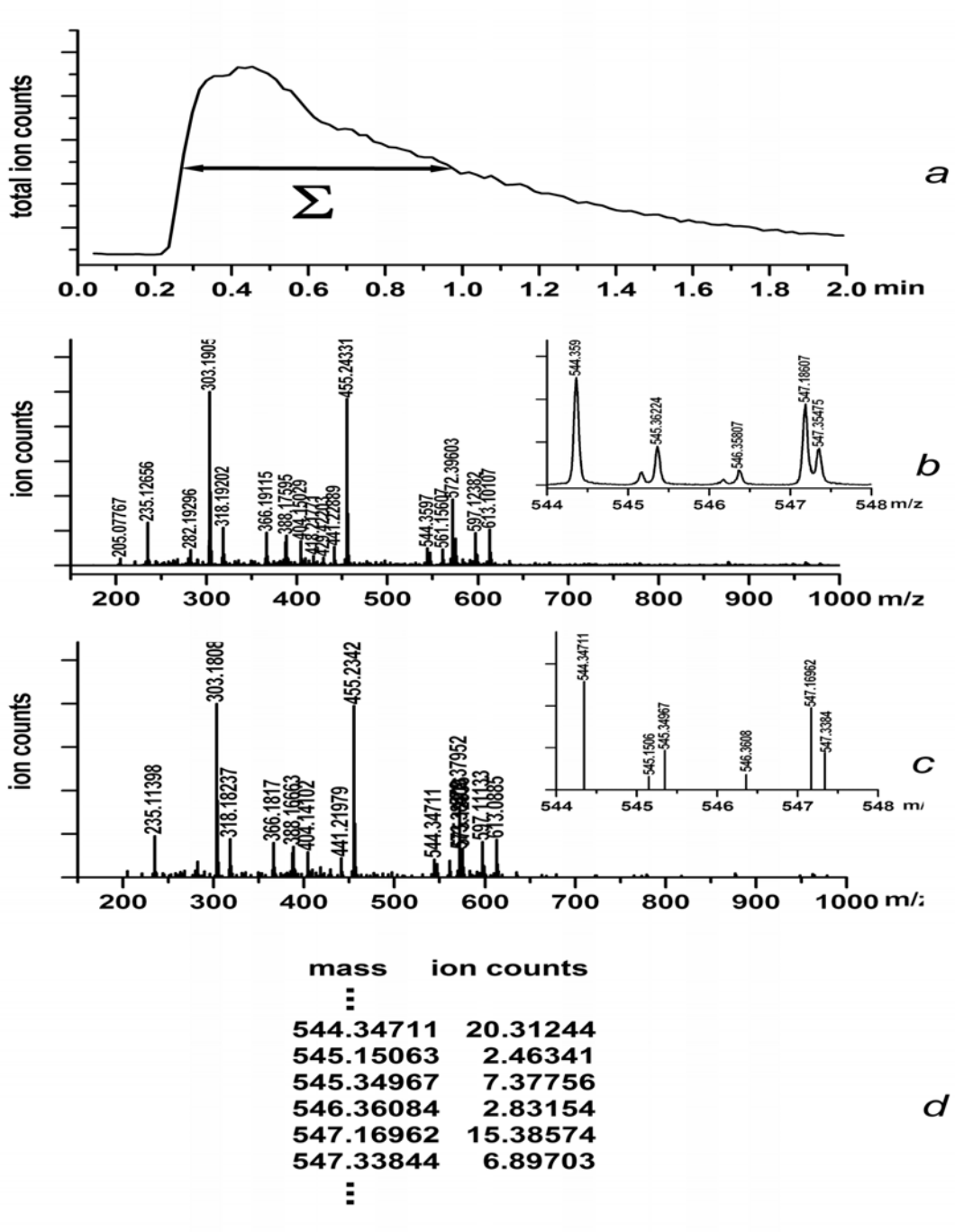

Fig. 1. Example of the raw data structure and processing from direct infusion ES-MS of a crude extract using *Penicillium viridicatum* as an example. From the elution profile a) about 60 scans are summarized into a continuum spectrum b) from which the centroid mass spectrum is calculated (centroid at 80% Full Width Half Maximum using viridic acid for internal mass correction see table 1) c). Finally the data are collected in a table) for further processing in this case 172 mass-ion count pairs. d. A small section is magnified to show the data structure.

Also, a cluster analysis will always place a sample somewhere, thus an odd (unique) isolate may end up in a cluster as an alien, although this will require a certain similarity with its neighbours. If we look at the YES data we find two clusters where all isolates of the given species are found but with an alien isolate: In the *P. hordei* cluster a single *P. tulipae* isolate is found and in the *P. echinulatum* cluster a single *P. commune* isolate is found. If we accept these still rather strict criteria 70 % of the species are classified correctly by the data from YES and 46% of the data from CYA. We find that this is in agreement with our previous studies (Smedsgaard & Frisvad 1997) as the present study includes more species and the isolates were selected to give a broader representation of the species. Although the analytical and data processing procedures have greatly improved, it has also exposed several contamination problems previous not seen (e.g. PEG from plastics).

In both dendrograms on figure 2 and 3 some clusters have been labelled by a letter as they cannot be described by a single or few species.

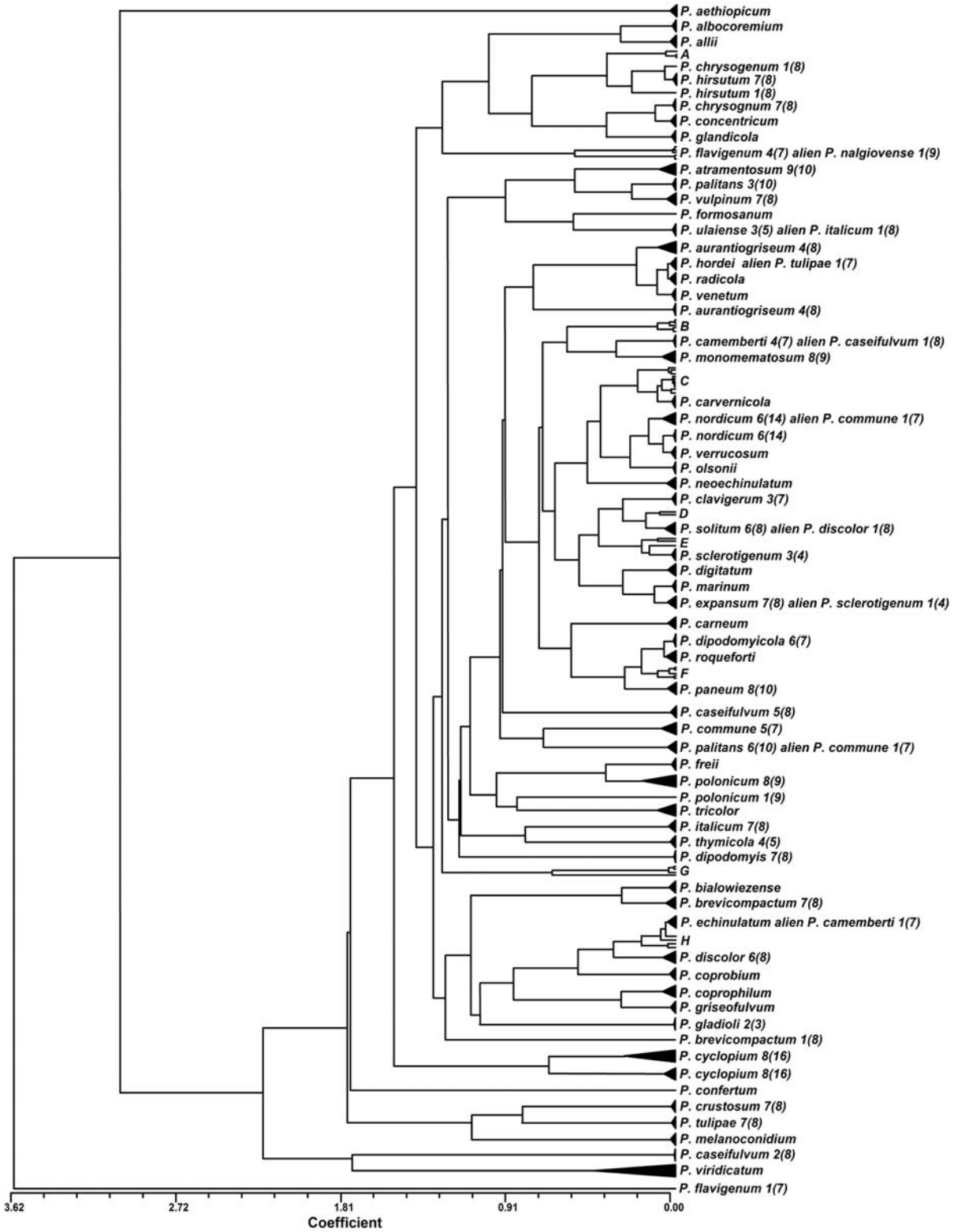

Fig. 2 Complete dendrogram from cultures grown on YES. Cluster labelled A to H are single isolates or isolates not forming any clear clusters. Numbers after the species name tells how many isolates are found in that cluster with the total number of isolate examined in brackets.

In a detailed analysis of the specific clusters we find that some species are divided in two groups, e.g. six of the seven *P. flavigenum* isolates are separated into the clusters labelled A and B with a significant outlier in the YES data (figure 2), whereas five isolates of *P. flavigenum* are found in a distinct cluster in the CYA data (figure 3) with the two remaining isolates in the cluster labelled K.

By both analyses an alien *P. italicum* isolate occurs in cluster of the closely related *P. ulaiense*, findings that are in agreement with those of (Frisvad and Samson 2004).

In the cluster group labelled C in the YES data (figure 2) seven of the nine *P. nalgiovense* isolates were found together with isolates of the related species: *P. camemberti* (1) and *P. solitum* (2). However, the *P. nalgiovense* isolates are split in two, five *P.*

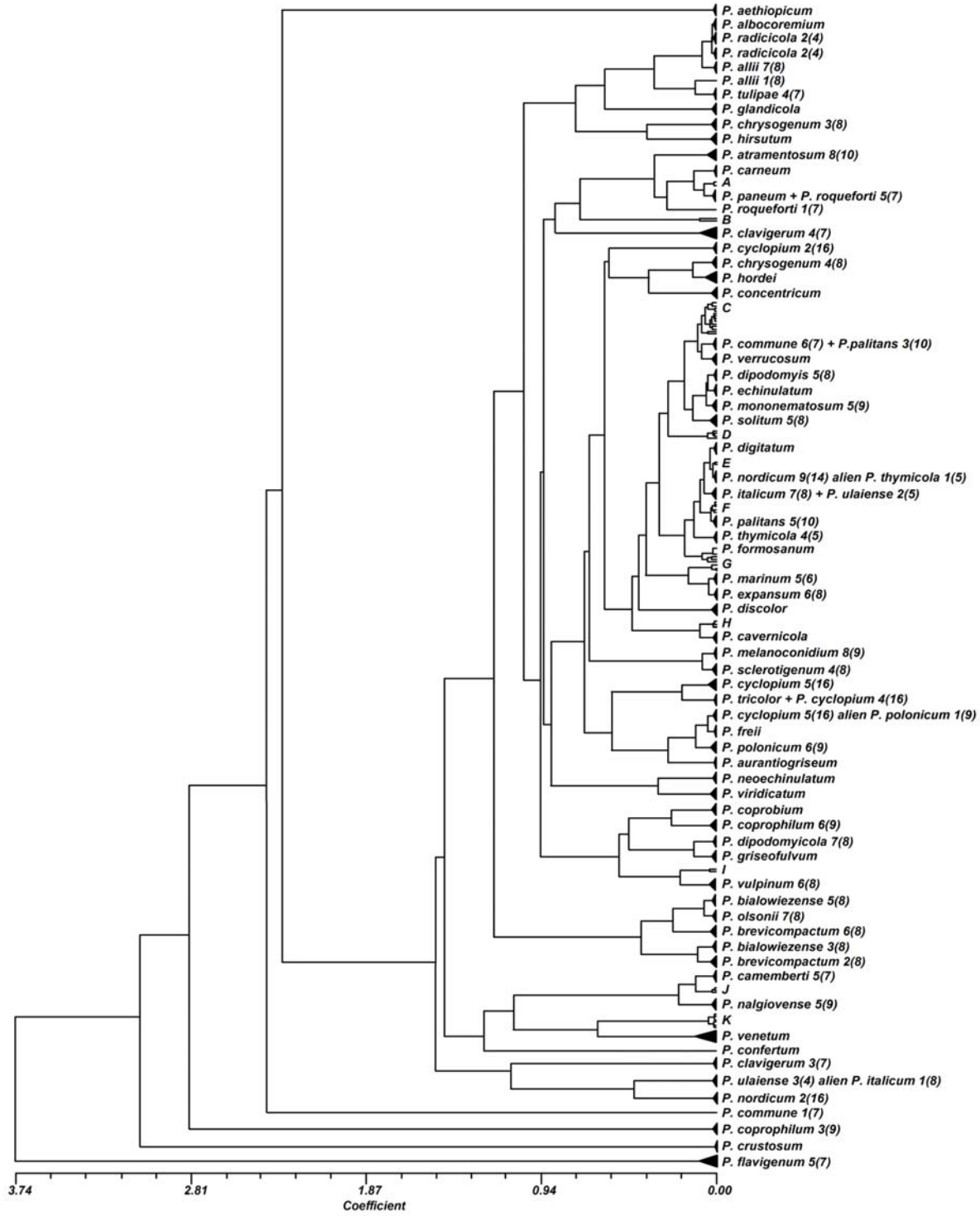

Fig. 3 Complete dendrogram from cultures grown on CYA. Clusters labelled A to K are single isolates or isolates not forming any clear clusters. Numbers after the species name tells how many isolates are found in that cluster with the total number of isolate examined in brackets.

nalgiovense and the two *P. solitum* is closer to *P. cavernicola* than the two remaining *P. nalgiovense* and the *P. camemberti*. The remaining two *P. nalgiovense* isolates are found in a cluster of isolates with a very low abundance of ions.

Analysing the YES data (figure 2) shows that *P. nordicum* is close to the related *P. verrucosum*, most noticeable divided into two separate clusters indicating two chemotypes. By the CYA data (figure 3) *P. nordicum* is distinctly separated from *P. verrucosum*, however 5 of the 14 included isolates are quite separate from the others. By analysing the CYA data (figure 3) most isolates of the two related species *P. paneum* and *P. roqueforti* are found in a single cluster, close together with another related species *P. carneum*. These three species constitute the section *Roqueforti*. It also shows that all taxa from series *Olsonii*, *P. brevicompactum*, *P. bialowiezense* and *P. olsonii* are found in a common cluster.

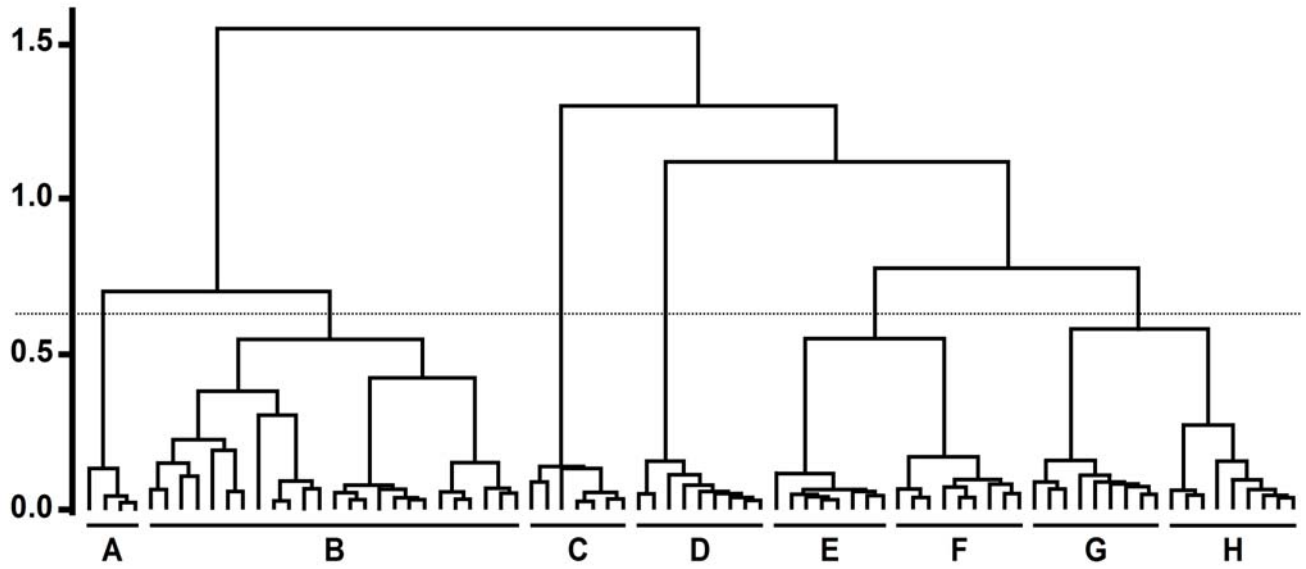

Fig. 4 Analysis of the series *Viridicata* alone gives a perfect classification in full agreement with recent classification (Frisvad and Samson 2004). A: *P. tricolor*, B: *P. cyclopium*, C: *P. viridicatum*, D: *P. melanoconidium*, E*: P. neoechinulatum*, F: *P. freii*, G: *P. aurantiogriseum*, H: *P. polonicum.*

P. aethiopicum is distinctly different from other species in both analyses and is also is accommodated in a separate series by Frisvad and Samson (2004).

Most isolate from five of the species in the series *Viridicata* are found in a common cluster in the CYA data, whereas these species are more divided by the YES data, particularly *P. aurantiogriseum* which is split into two clusters in the YES data (figure 2)

As the classification is based solely on chemical data one may ask the question what is bringing these clusters together. This is not an easy question to answer as any cluster analysis including the AMS distance will include all variables in this case 100 to 600 ions detected in each spectrum. However some metabolites produce quite significant ions in the spectra e.g. meleagrin and several of the species producing meleagrin will do so in quite large amounts. These species also produce roquefortine C, another metabolite from the same pathway (Frisvad et al. 2004). Therefore a cluster grouping half the meleagrin producing species, all which are very good producers can be located near the top in dendrograms from both YES and CYA.

In the YES dendrogram (figure 2) the species *P. albocoremium*, *P. allii*, *P. chrysogenum*, *P. hirsutum*, *P. concentricum* and *P. glandicola* are grouped most likely due to meleagrin and roquefortine C. The species in the cluster labelled A is also producing meleagrin. In the CYA dendrogram (figure 3) *P. albocoremium*, *P. radicicola*, *P. allii*, *P. tulipae*, *P. glandicola*, *P. chrysogenum* and *P. hirsutum* are group by melgarin and roquefortine C production. Even though these metabolites produce quite significant ions, there is still enough other information in the spectra to separate most of these species. The remaining meleagrin-roquefortine C producing species is distributed throughout the dendrograms.

The direct infusion ESMS methodology also presents some evidence on existence of chemical series as compared to natural series as presented by Frisvad and Samson (2004). In some case these may be quite similar, where in other cases the can be quite different. The species in the series *Corymbifera* with the exception of *P. hordei,* all produce meleagrin and most of these are found in the meleagrin cluster described above in the CYA data (figure 3). *P. venetum,* a rather poor producer, of meleagrin is located in a unique cluster elsewhere as is the non meleagrin producing *P. hordei*. In the YES data (figure 2) the taxa of the series *Corymbifera* are split into two clusters with the other meleagrin producing species.

Another interesting observation is that several of the coprophilic species *P. coprobium*, *P. coprophilium*, *P. griseofulvum*, *P. vulpinum* are grouped with one or two characteristic soil species.

If a smaller group of species is of interest and this group can be delimited by other means, it is possible to perform a much clearer cluster analysis. Figure 4 illustrate this by a cluster analysis of a subset of the data selecting 80 isolates from the 8 species in the series *Viridicata*, generally considered as difficult to identify. In this case the classification is a perfect clustering of all isolates into correct species. Furthermore, a separation of *P. cyclopium* into the two taxa: *P. cyclopium* and *P. aurantiovirens* (Lund & Frisvad 1994) is supported only by these chemical data.

The major advantage of direct infusion electrospray mass spectrometry is that a non biased chemical profile can be determined in minutes without requirement of metabolite knowledge. Also the samples can in most cases be prepared directly from the normal seven days old cultures using only one medium. This study does not conclude which medium is pref-

erable for automated classification. At first glance the data from YES separate most species into correct taxa and do in general increase the amount of metabolites produced (not published observations). However, for most of these species CYA will give a higher chemical diversity which in case will result in a more branched dendrogram. The use of direct positive electrospray mass spectrometry methodology favours alkaloids thus species producing unique or significant alkaloids will be more efficiently classified compared to species characterised by e.g. polyketides. To compensate for this bias a careful balance between the qualitative and quantitative features of the spectra have to be selected. The developed data processing methodology allow almost automated processing weighting both features. As seen from the cluster analysis a major part of the examined species can be classified directly from the ESI-MS spectra. As mass spectra are ideal for storage in databases with automated search and retrieval this forms an efficient base for an automated identification system.

Acknowledgements
We thank the Danish Technical Research Council for support for Program for Predictive Biotechnology (Functional biodiversity in *Penicillium* and *Aspergillus*) and Centre for Advanced Food Studies (LMC), Denmark, for additional financial support. Furthermore Ellen Kirstine Lyhne and Hanne Jakobsen are greatful acknowledged for their assistance in the laboratory cutting plugs, extracting and analyzing the samples.

Note: Full dendrograms with culture references (IBT collection numbers) are available from the authors as pdf files.

Literature cited

Filtenborg O, Frisvad JC, (1980) A simple screening method for toxigenic moulds in pure cultures. *Lebensm Wiss Technol* **13:** 128-130.

Filtenborg O, Frisvad JC, Svendsen JA, (1983) Simple screening method for moulds producing intracellular mycotoxins in pure cultures. *Applied and Environmental Microbiology* **45:** 581-585.

Frisvad JC, (1987) High-performance liquid chromatographic determination of profiles of metabolites of mycotoxins and other secondary metabolites. *Journal of Chromatography A* **392:** 333-347.

Frisvad JC, (1994) Classification of organisms by secondary metabolites. In: *Identification and characterization of pest organisms*. (Hawksworth, D. L. ed.). CAB International, 303-320

Frisvad JC, Samson RA, (2004) Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of the food and air-borne terverticillate Penicillia and their mycotoxins. *Studies in Mycology* **49**: 1-173.

Frisvad JC, Smedsgaard J, Larsen TO, Samson R, (2004) Mycotoxins, drugs and other extrolites produced by species in the *Penicillium* subgenus *Penicillium*. *Studies in Mycology* **49**.

Frisvad JC, Thrane U, (1987) Standardized high-performance liquid chromatography of 182 mycotoxins and other fungal metabolites based on alkylphenone retention indices and UV-VIS spectra (diode array detection). *Journal of Chromatography A* **404:** 195-214.

Frisvad JC, Thrane U, (1993) Liquid column chromatography of mycotoxins. In: *Chromatography of mycotoxins-Techniques and applications*. (Betina, V. ed.). 1 edn. Elsevier Amsterdam, 253-372

Hansen ME, Smedsgaard J, (2004a) A New Matching Algorithm for High Resolution Mass Spectra. *Journal of the American Society of Mass Spectrometry*. in press

Hansen ME, Smedsgaard J, (2004b) Automated data processing of high-resolution mass spectra from direct infusion of complex fungal extracts. *Journal of the American Society of Mass Spectrometry*. in press

Lund F, Frisvad JC, (1994) Chemotaxonomy of *Penicillium auratiogriseum* and related species. *Mycological Research* **98:** 481-492.

Nielsen KF, Smedsgaard J, (2003) Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography-UV-mass spectrometry methodology. *Journal of Chromatography A* **1002:** 111-136.

Nielsen N-PV, Carstensen JM, Smedsgaard J, (1998) Aligning of single and multiple wavelength chromatographic profiles for chemometric data analysis using correlation optimised warping. *Journal of Chromatography A* **805:** 17-35.

Nielsen N-PV, Smedsgaard J, Frisvad JC, (1999) Full second-order chromatographic/spectrometric data matrices for automated sample identification and componenet analysis by non-data-reducing image analysis. *Analytical Chemistry* **71:** 727-735.

Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O, (2004) *Introduction to food-borne fungi.* 7th edn. Baarn, The Netherlands, Centraalbureau voor Schimmelcultures.

Smedsgaard J, (1997a) Micro-scale extraction procedure for standardized screening of fungal metabolite production in cultures. *Journal of Chromatography A* **760:** 264-270.

Smedsgaard J, (1997b) Terverticillate penicillia studied by direct electrospray mass spectrometric profiling of crude extracts: II. Database and identification. *Biochemical Systematics and Ecology* **25:** 65-71.

Smedsgaard J, Frisvad JC, (1996) Using direct electrospray mass spectrometry in taxonomy and secondary metabolite profiling of crude fungal extracts. *Journal of Microbiological Methods* **25:** 5-17.

Smedsgaard J, Frisvad JC, (1997) Terverticillate penicillia studied by direct electrospray mass spectrometric profiling of crude extracts: I. Chemosystematics. *Biochemical Systematics and Ecology* **25:** 51-64.

Svendsen A, Frisvad JC, (1994) A chemotaxonomic study of the terverticillate penicillia based on high performance liquid chromatography of secondary metabolites. *Mycological Research* **98:** 1317-1328.