Classification of some dermatophytes by pyrolysis–gas–liquid chromatography[1]

J. W. CARMICHAEL, AWATAR S. SEKHON, AND LYNNE SIGLER
University of Alberta Mold Herbarium and Culture Collection, Edmonton, Alberta

Accepted December 6, 1972

CARMICHAEL, J. W., A. S. SEKHON, and L. SIGLER. 1973. Classification of some dermatophytes by pyrolysis–gas–liquid chromatography. Can. J. Microbiol. **19**: 403–407.

Samples from dried colonies of 21 strains of *Nannizzia* and *Arthroderma* were analyzed by pyrolysis–gas–liquid chromatography. Characteristic peak patterns produced by all the strains were used as markers to correct random drift in retention time so that corresponding peaks in different pyrograms could be homologized. Variation in sample size was compensated for by comparing peaks on each pyrogram with a particular major component and scoring them simply as 0 (absent), 1 (small), or 2 (large). Proximities were calculated and analyzed for clusters by the TAXMAP procedure. The analysis always grouped replicate samples together in the same cluster. Opposite mating types of the same species were sometimes placed in the same cluster and sometimes in separate clusters. The (+) mating type of *Arthroderma benhamiae* was placed in a cluster with both mating types of *Nannizzia gypsea* and *N. obtusa*, while the (−) mating type replicates of *A. benhamiae* were placed in a cluster by themselves. Finding a greater difference between pyrograms of different mating types of one species than between pyrograms of different species was unexpected and requires further investigation.

CARMICHAEL, J. W., A. S. SEKHON et L. SIGLER. 1973. Classification of some dermatophytes by pyrolysis–gas–liquid chromatography. Can. J. Microbiol. **19**: 403–407.

Des échantillons de colonies séchées de 21 lignées de *Nannizzia* et d'*Arthroderma* furent analysés par chromatographie en pyrolyse gaz–liquide. Des modèles de pics caractéristiques produits par toutes les lignées furent utilisés comme marqueurs pour corriger l'écart au hazard dans le temps de retention de telle sorte que les pics correspondants chez différents pyrogrames puissent être homologués. La variation dans la grosseur de l'échantillon fut compensée en comparant les pics de chaque pyrograme avec un composant majeur particulier et en les évaluant simplement comme 0 (absent), 1 (petit) et 2 (large). Les rapprochements furent calculés et analysés pour les clônes par la procédure TAXMAP. Les analyses ont toujours groupé ensemble les replicats des échantillons dans le même clône. Les types conjugaux opposés de la même espèce furent quelquefois placés dans le même clône, et quelquefois dans des clônes séparés. Le type conjugal (+) d'*Arthroderma benhamiae* fut placé dans un clône avec les deux types conjugaux de *Nannizzia gypsea* et *N. obtusa*, alors que les replicats d'*A. benhamiae* du type conjugal (−) furent placés dans un clône par eux-mêmes. Il était inattendu de trouver une plus grande différence entre les pyrogrames de différents types conjugaux d'une espèce, qu'entre les pyrogrames d'espèces différentes; cette observation demande une étude plus poussée. [Traduit par le journal]

Can. J. Microbiol. Downloaded from www.nrcresearchpress.com by University of Alberta on 02/05/15
For personal use only.

Introduction

Pyrolysis–gas–liquid chromatography (PGLC) has been proposed as a method for obtaining data-rich 'fingerprints' which could be used to identify complex organic materials or even whole organisms (Reiner 1965, 1967; Oyama and Carle 1967). The technique has been applied to taxonomic studies of green plants (Harley and Bell 1967), bacteria (Reiner *et al.* 1969; Cone and Lechowich 1970), and fungi (Vincent and Kulik 1970).

In a preliminary study of factors affecting chromatograms of dermatophyte fungi (Gymnoascaceae), we found that a low resolution chromatographic system was not suitable for classification (Sekhon and Carmichael 1972). It has been shown by mass spectrometry that single peaks of a pyrogram may be composed of a mixture of several different pyrolysis fragments (Simmonds 1970). In our study, the way these fragments combined to produce peaks in pyrograms from similar strains or replicate samples apparently caused us to make errors in selecting homologous peaks for taxonomic comparison. By changing chromatographic columns and temperature programming, we have been able to achieve a threefold increase in the number of peaks resolved from samples prepared and pyrolyzed in the same way as before. This paper presents the results of a study of 21 strains of dermatophytes using the improved technique.

Materials and Methods

Organisms

Plus and minus mating types of *Arthroderma benhamiae* and of seven species of *Nannizzia* were analyzed. Also included were two strains of *Microsporum audouini*, and one strain each of *Microsporum* sp., *M. canis*, and *Trichophyton* sp. Details of the strains are given in Table 1. Most of the strains were obtained from the Commonwealth Mycological Institute (CMI), Kew, England,

[1]Received September 5, 1972.

Can. J. Microbiol. Downloaded from www.nrcresearchpress.com by University of Alberta on 02/05/15
For personal use only.

or the National Communicable Disease Center (NCDC), Atlanta, Georgia, U.S.A.

Culture Medium and Growth Conditions

Cultures were grown at 25C on squares of cellophane placed on plates of cereal agar medium (Pablum precooked mixed cereal, 100 g; agar, 15 g; and deionized distilled water, 1 liter). The procedures for growing, harvesting, and storing the fungal colonies were the same as described previously (Carmichael 1963; Sekhon and Carmichael 1972). Three or four 3-week-old colonies were prepared for each strain at the beginning of the study, thus providing a surplus of material for pyrolysis. Samples prepared from the colonies of any one strain are referred to as replicate samples.

Chromatography

Samples for PGLC analysis were prepared by suspending a colony in water–acetone–ethanol mixture and introducing the resulting slurry into short capillary tubes. After evaporation of the solvent, capillary tubes were placed inside the pyrolyzer coil for pyrolytic degradation. After pyrolysis, the volatile products were swept into the chromatographic column in a Carlo Erba Fractovap, model GV chromatograph, equipped with a flame-ionization detection unit. The columns used were 2.4 m × 5 mm internal diameter, and contained 4% Carbowax 20M, TPA, coated on chromosorb G, AW-DCMS, 60/70 mesh. They were temperature-programmed from 50 to 225C with a linear rise of 4C per minute. Other details of the procedures were described previously (Sekhon and Carmichael 1972).

TABLE 1

Details of strains studied

UAMH	Name and mating type	Source
1485	*Nannizzia gypsea* +	CMI as 86175, single-ascospore isolate from 80558, rec'd. 1962
1486	*N. gypsea* −	CMI as 86176, single-ascospore isolate from 80558, rec'd. 1962
1465	*N. grubia* +	NCDC as X-470, rec'd. 1962
1466	*N. grubia* −	NCDC as X-471, rec'd. 1962
1481	*N. fulva* +	CMI as 86179, single-ascospore isolate, rec'd. 1961
1480	*N. fulva* −	CMI as 86180, single-ascospore isolate, rec'd. 1961
1512	*N. obtusa* +	CMI as 84309, rec'd. 1961
1482	*N. obtusa* −	CMI as 84310, rec'd. 1961
2940	*N. persicolor* +	CMI as 117073, rec'd. 1968
2939	*N. persicolor* −	CMI as 117064, rec'd. 1968
2937	*N. cajetani* +	CMI as 117057 (=ATCC 14387), rec'd. 1968
2938	*N. cajetani* −	CMI as 117058 (=ATCC 14388), rec'd. 1968
2936	*N. incurvata* +	CMI as 86523, rec'd. 1968
2935	*N. incurvata* −	CMI as 86518, rec'd. 1968
2822	*Arthroderma benhamiae* +	NCDC as X-797, rec'd. 1967
2823	*A. benhamiae* −	NCDC as X-798, rec'd. 1967
905	*Microsporum audouini*	Hair, 1961, Edmonton, Alberta
912	*M. audouini*	Hair, 1961, Edmonton, Alberta
2338	*M. canis*	Hair, 1965, Edmonton, Alberta
2389	*Microsporum* sp.	A. K. Garg, Univ. of Rajasthan, as No. 585, from soil, rec'd. 1965
2733	*Trichophyton* sp.	C. Magalhaes, Mozambique, as No. 1044, from soil, rec'd. 1967

Scaling and Recording Pyrogram Data

The first 48 min of the retention time scale was divided into 240 units of 12 s each. Visual examination showed that characteristic peaks were present in all the pyrograms at about 8, 22, 24, 37, and 39 min retention time. Comparison of pyrograms from replicate samples showed that there was some random drift in retention time along the pyrogram curves. Therefore, the characteristic peaks were used as markers to correct the retention time scale so that homologous intermediate peaks on different pyrograms could be numbered with the same retention time unit. A total of 114 peaks were resolved, as compared with 37 in our previous study, and problems of drift and homology determination were greatly reduced. Replicate samples from a check strain were pyrolyzed at intervals during the course of the study. The resulting pyrograms were compared visually with one produced at the beginning. No change in column performance was noted by this procedure.

The data was recorded using two procedures to compensate for sample size variation. First, the 20 highest peaks of each pyrogram were recorded in rank order. Since there did not appear to be any shift in base line during each run, peak height was simply read from the detector response scale on the chart paper. It was hoped that the major components of pyrolysis contained most of the taxonomically useful information, and that this simple and economical procedure would be satisfactory. Second, peak heights were recorded on a 0–1–2 scale. The height of peak 40 was taken as a measure of sample size and used to set the dividing line for recording the other peaks as either 1 or 2. Missing peaks were scored 0.

The recorded data were entered onto disk files through a terminal and analyzed for relative proximities and clusters using the TAXMAP classification program of Carmichael and Sneath (1969) and Carmichael (1970). The analysis included a total of 105 pyrograms. The number of replicate pyrograms for each strain is given in parentheses after the strain numbers in Fig. 1.

Results and Discussion

The rank order of the 20 highest peaks varied considerably from one replicate sample to another. When the peaks were weighted by their rank order, the cluster analysis placed some replicates of eight of the species in one cluster, and distributed the remaining pyrograms into 38 small clusters, mostly with one to three

Can. J. Microbiol. Downloaded from www.nrcresearchpress.com by University of Alberta on 02/05/15
For personal use only.

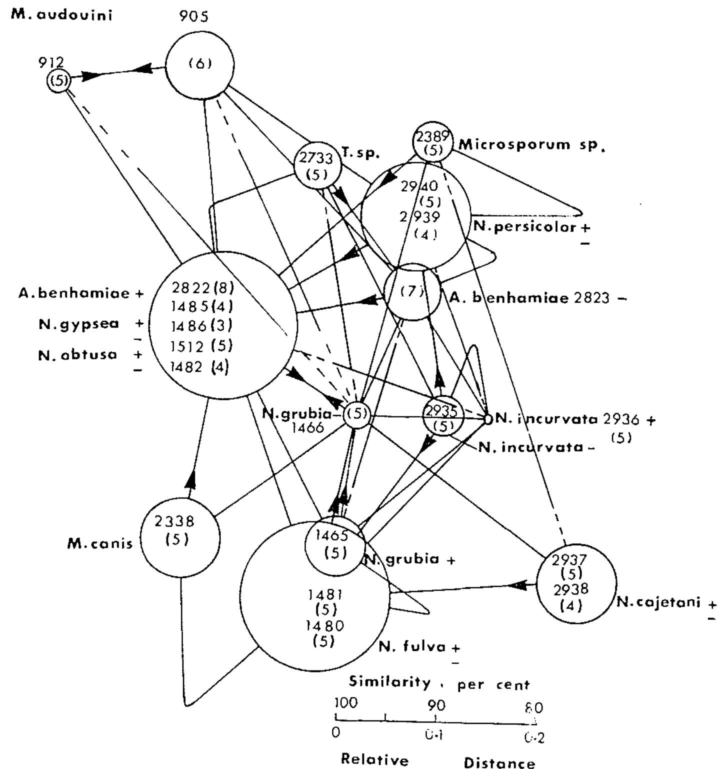


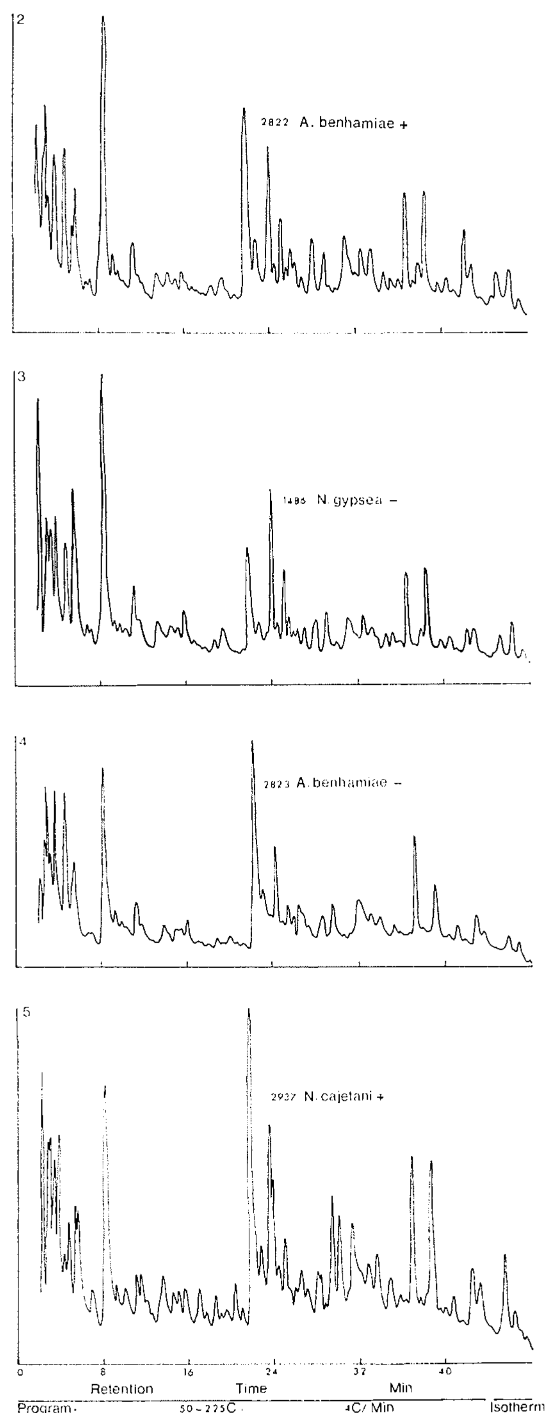
FIG. 1. Taxometric map of the relations among 21 strains of dermatophytes based on pyrogram data. The diameter of the circles represents the maximum distance between any pair of samples included in the cluster. The numbers in parentheses after the strain numbers give the number of replicate samples of each strain. The lines connecting the margins of the circles represent the distance between the nearest neighbors in the two clusters. Each cluster is shown the proper distance from its two nearest neighbors. To fit the clusters into two dimensions, other distances may be distorted, as indicated by bent lines of the appropriate length and by straight lines of the appropriate length with dashed continuations. The arrows indicate the nearest neighbor to each cluster. Strain 2935 was equidistant from 1481 and 2940, and thus has two arrows.

members in each. When the 19 highest peaks on each pyrogram were recorded simply as present, and the remaining peaks as absent, the cluster analysis was slightly improved, but still very unsatisfactory. It appears that selecting only the major components is not a suitable short-cut for comparing pyrograms.

When the pyrograms were analyzed with the data obtained by scoring all 114 of the peaks on the 0–1–2 scale, an interesting classification resulted. The taxometric map for this classification is shown in Fig. 1. With this procedure, replicate samples were always placed in the same cluster. The variation among replicates was small, mostly zero to 0.04 distance units (which is equivalent to a similarity of 100 to 96%). The least similar pair of replicate pyrograms had a relative distance of 0.11 (similarity = 89%). The clusters were well separated, compared to the variation within them, indicating a stable classification. Only one cluster contained strains belonging to more than one species. For five of the species, the (+) and (−) mating types were included in the same cluster. For three of the species, they were placed in separate clusters.

At present, it is not possible to identify (+) and (−) mating types except by mating tests. However, it has been shown that the mating types of pathogenic species differ in virulence (Rippon and Garber 1969; Rippon 1971). In some species, we have observed that strains of one mating type tend to be more granular and more pigmented than strains of the other mating type. According to the pyrograms of the strains included in this study, there is a wide range of variation between mating types in the different

Can. J. Microbiol. Downloaded from www.nrcresearchpress.com by University of Alberta on 02/05/15
For personal use only.



FIGS. 2–5. Pyrograms of strains from the same cluster (Figs. 2 and 3), and different clusters (Figs. 4 and 5). The vertical axis represents linear detector response.

species. The two mating types were very similar in *N. cajetani*, *N. gypsea*, and *N. obtusa*. They were less similar in *N. persicolor* and *N. fulva*. In *N. incurvata*, *N. grubia*, and *A. benhamiae*, the two mating types fell in different clusters. It is especially surprising that the (+) mating type of *A. benhamiae* was so close to the (−) mating type of *N. gypsea* that they fell in the same cluster. The (−) mating type replicates of *A. benhamiae* formed a separate cluster whose nearest neighbors were replicates of *N. gypsea* and *N. obtusa*, not its own opposite mating type. Figures 2 and 3 show the very similar pyrograms for a replicate of *A. benhamiae* (+) and of *N. gypsea* (−). Figure 4 shows a replicate of *A. benhamiae* (−) which differs from the preceding two. Figure 5 shows a pyrogram for *N. cajetani*, as an example of a relatively distant species.

Nannizzia gypsea, *N. fulva*, and *N. incurvata* are very similar to each other in morphology. Their conidial states are usually identified only as belonging to the *Microsporum gypseum* group, unless mating tests are done (Weitzman *et al.* 1967). It is surprising therefore to find that the pyrogram data places *N. gypsea* in a cluster with the morphologically distinct *N. obtusa* and with *A. benhamiae*, while *N. fulva* and *N. incurvata* are in separate clusters.

The unidentified *Microsporum* (2389) was originally identified as an atypical *M. canis* on morphological grounds. Subsequently, Padhye (1971) reported that it produced ascocarps when mated with a (−) mating-type strain of *N. cajetani* (2938). Its placement on the taxometric map is quite distant from both *M. canis* and from the *N. cajetani* cluster. It may represent an undescribed species or a markedly changed mutant.

The unidentified *Trichophyton* (2733) was at first identified as *Trichophyton indicum*, which is now known to be the conidial state of *Aphanoascus terreus* (Randhawa and Sandhu) Apinis (Apinis 1968). Its closeness to *Arthroderma benhamiae* on the map suggests that it more likely belongs to the *T. terrestre* group of Trichophytons, several of which are known to have an *Arthroderma* perfect state.

The two strains of *M. audouini* are fairly close to each other and well separated from the other species, including *M. canis*. This is interesting in view of our previous results with these

Can. J. Microbiol. Downloaded from www.nrcresearchpress.com by University of Alberta on 02/05/15
For personal use only.

strains, where they appeared to be quite different on the basis of low-resolution pyrograms (Sekhon and Carmichael 1972). We also had trouble previously with the replicates of these strains showing excessive variation, whereas in this set of pyrograms, the least similar replicates of 912 were 98% similar and the least similar replicates of 905 were 94% similar.

Conclusions

Pyrograms can provide data suitable for taxonomic studies if the variation between replicate pyrograms can be controlled within close limits, and if the resolution of the system is sufficient to avoid major problems in determining the homologies between peaks of different strains. For the moment, this limits comparison with samples run on the same set of columns and probably on the same chromatograph. The main limiting factor for comparing different runs appears to be the lack of a technique for producing standardized columns.

The classification produced from the pyrogram data on our dermatophyte strains does not agree with the traditional classification of this group. Additional studies are needed to explore the relation between pyrolysis patterns and conventional classifications.

Acknowledgment

This research was supported in part by a grant from the Medical Research Council, Ottawa, Canada.

Apinis, A. E. 1968. Relationships of certain keratinophilic Plectascales. Mycopath. Mycol. Appl. **35**: 97–104.

Carmichael, J. W. 1963. Dried mold colonies on cellophane. Mycologia, **55**: 283–288.

——— 1970. The TAXMAP classification program. Univ. Alberta Mold Herb. mimeogr., Edmonton, Alberta.

Carmichael, J. W., and P. H. A. Sneath. 1969. Taxometric maps. Syst. Zool. **18**: 402–415.

Cone, R. D., and R. V. Lechowich. 1970. Differentiation of *Clostridium botulinum* types A, B, and E by pyrolysis–gas–liquid chromatography. Appl. Microbiol. **19**: 138–145.

Harley, R. M., and M. G. Bell. 1967. Taxonomic analysis of herbarium material by gas chromatography. Nature (London), **213**: 1241–1242.

Oyama, V. I., and G. C. Carle. 1967. Pyrolysis–gas chromatography application to life detection and chemotaxonomy. J. Gas Chromatogr. **5**: 151–154.

Padhye, A. A. 1971. An unusual variant of *Microsporum cookei*. Sabouraudia, **9**: 254–255.

Reiner, E. 1965. Identification of bacterial strains by pyrolysis–gas–liquid chromatography. Nature (London), **206**: 1272–1274.

——— 1967. Studies on differentiation of microorganisms by pyrolysis–gas–liquid chromatography. J. Gas Chromatogr. **5**: 65–67.

Reiner, E., R. E. Beam, and G. P. Kubica. 1969. Pyrolysis–gas–liquid chromatography studies for the classification of mycobacteria. Am. Rev. Respir. Dis. **99**: 750–759.

Rippon, J. W. 1971. Differences between '+' and '−' strains of keratinophilic fungi. *In* Recent advances in microbiology. *Edited by* Perez-Miravete and Pelaez. Int. Congr. Microbiol., Mexico, D. F. pp. 473–475.

Rippon, J. W., and E. D. Garber. 1969. Dermatophyte pathogenicity as a function of mating type and associated enzymes. J. Invest. Dermatol. **53**: 445–448.

Sekhon, A. S., and J. W. Carmichael. 1972. Pyrolysis–gas–liquid chromatography of some dermatophytes. Can. J. Microbiol. **18**: 1593–1601.

Simmonds, P. C. 1970. Whole microorganisms studied by pyrolysis–gas chromatography – mass spectrometry: significance for extraterrestrial life detection experiments. Appl. Microbiol. **20**: 567–572.

Vincent, P. G., and M. M. Kulik. 1970. Pyrolysis–gas–liquid chromatography of fungi: differentiation of species and strains of several members of the *Aspergillus flavus* group. Appl. Microbiol. **20**: 957–963.

Weitzman, I., M. Silva-Hutner, and I. Kozma. 1967. A comparison of the pathogenicity of three members of the *Microsporum gypseum* complex. Sabouraudia, **5**: 360–365.

This article has been cited by:

1. Chapter 17. Analytical pyrolysis of microorganisms 471-483. [CrossRef]
2. Michael Goodfellow, Roger Freeman, Penelope R. Sisson. 1997. Curie-Point Pyrolysis Mass Spectrometry as a Tool in Clinical Microbiology. *Zentralblatt für Bakteriologie* **285**, 133-156. [CrossRef]
3. G. Almendros, A.T. Martínez, A.E. González, F. Martín, F.J. González-Vila. 1990. Pyrolysis-gas chromatography-mass spectrometry of the yeast genera Cryptococcus and Rhodotorula. *Mycological Research* **94**, 211-218. [CrossRef]
4. Tadahiko Matsumoto, Libero Ajello. 1987. Current Taxonomic Concepts Pertaining to the Dermatophytes and Related Fungi. *International Journal of Dermatology* **26**:10.1111/ijd.1987.26.issue-8, 491-499. [CrossRef]
5. P.H.A. SNEATHFuture of Numerical Taxonomy 415-431. [CrossRef]
6. C.S. GUTTERIDGE, L. VALUS, H.J.H. MacfieNumerical Methods in the Classification of Micro-organisms by Pyrolysis Mass Spectrometry 369-401. [CrossRef]
7. G. L. FRENCH, C. S. GUTTERIDGE, I. PHILLIPS. 1980. Pyrolysis Gas Chromatography of Pseudomonas and Acinetobacter Species. *Journal of Applied Bacteriology* **49**:10.1111/jam.1980.49.issue-3, 505-516. [CrossRef]
8. C. Madhosingh. 1980. Fusarium oxysporum f. sp. spinaciae. A Biochemical Comparison of Race 1 and Race 2. *Journal of Phytopathology* **98**, 27-39. [CrossRef]
9. F.J. Swenson, J.A. Ulrich. 1980. Fatty acids of dermatophytes. *Medical Mycology* **18**, 1-9. [CrossRef]
10. E. Reiner, L. E. Abbey, T. F. Moran, P. Papamichalis, R. W. Schafer. 1979. Characterization of normal human cells by pyrolysis gas chromatography mass spectrometry. *Biological Mass Spectrometry* **6**:10.1002/bms.v6:11, 491-498. [CrossRef]
11. W.J. Irwin. 1979. Analytical pyrolysis—an overview. *Journal of Analytical and Applied Pyrolysis* **1**, 89-122. [CrossRef]
12. C. S. GUTTERIDGE, J. R. NORRIS. 1979. The Application of Pyrolysis Techniques to the Identification of Micro-organisms. *Journal of Applied Bacteriology* **47**:10.1111/jam.1979.47.issue-1, 5-43. [CrossRef]
13. C.S. Gutteridge, H.J.H. MacFie, J.R. Norris. 1979. Use of principal components analysis for displaying variation between pyrograms of micro-organisms. *Journal of Analytical and Applied Pyrolysis* **1**, 67-76. [CrossRef]
14. W.J. Irwin. 1979. Analytical pyrolysis - an overview. *Journal of Analytical and Applied Pyrolysis* **1**, 3-25. [CrossRef]
15. James D. Brosseau, J. W. Carmichael. 1978. Pyrolysis gas-liquid chromatography applied to a study of variation in Arthroderma tuberculatum. *Mycopathologia* **63**, 67-79. [CrossRef]
16. G.S. Oxborrow, N.D. Fields, J.R. PuleoPYROLYSIS GAS-LIQUID CHROMATOGRAPHY STUDIES OF THE GENUS BACILLUS. EFFECT OF GROWTH TIME ON PYROCHROMATOGRAM REPRODUCIBILITY 69-76. [CrossRef]
17. A.S. Sekhon, J.W. Carmichael. 1975. Classification of some gymnoascaceae by pyrolysis-gas-liquid chromatography using added marker compounds. *Medical Mycology* **13**, 83-88. [CrossRef]

Can. J. Microbiol. Downloaded from www.nrcresearchpress.com by University of Alberta on 02/05/15
For personal use only.