Chemotaxonomic Discrimination of Lichens by ^{1}H NMR, solution and HR-MAS, and Chemometric Analysis

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Abstract: *Lichens present a difficult morphological differentiation, and chemical analysis are often used for their taxonomic classification, mainly because secondary metabolites are relatively constant for these organisms. Information on metabolic composition can be easily obtained by ^{1}H NMR, although chemometric analysis of spectral data is required. This work focuses on the application of solution and ^{1}H HR-MAS NMR and chemometric analysis to the chemotaxonomic discrimination of lichens. The combination of these techniques shows to be effective to identify different lichens and discriminate families, genera and species.*

Resumo: *Os liquens apresentam uma difícil diferenciação morfológica, assim as análises químicas são muito empregadas para a classificação taxonômica, principalmente devido aos metabólitos secundários serem relativamente constantes para esses organismos. Desta forma, a informação sobre a composição metabólica pode ser facilmente obtida pelos espetros de RMN de ^{1}H, embora o uso de análises quimiométricas seja requrido. Este trabalho foca a aplicação de RMN de ^{1}H em solução e HR-MAS, associada às análises quimiométricas para a quimiotaxonomia de liquens. A combinação destas técnicas foi efetiva para identificar diferentes liquens e discriminar famílias, gêneros e espécies.*

Introduction

Lichens are found in a variety of habitats including those with extreme environmental conditions.[1,2] These organisms have morphological similarities, which makes taxonomic classification based on morphological differentiation complicated. In this respect, chemotaxonomy has been an important contribution, as the content of metabolites is considered relatively constant for similar species.[3] Therefore, chemical analysis of lichen taxonomy has been commonly used. Lichens are traditionally identified by several analyses such as color reactions, micro-crystallization, Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS).[4] However, information on metabolic composition can be easily obtained by ^{1}H NMR spectra.

The literature has reported on the use of ^{13}C NMR spectra of polysaccharides for lichen identification, and ^{13}C NMR spectra of glucans have been suggested as a chemotaxonomic key.[5-9] However, for chemotaxonomic purposes, using ^{1}H NMR spectra requires chemometric analysis of spectral data, which makes it easier to deal with the large amount of information.[10]

High Resolution Magic Angle Spinning (HR-MAS) NMR is used for the study of

heterogeneous samples and for direct analysis of animals and vegetable tissues[11,12] and foods.[13-16] The direct NMR analysis of these materials, even when solvated, is difficult due to restrict motion and physical heterogeneity of the sample, which can severely degrade spectrum resolution. Nevertheless, in HR-MAS NMR, typical features observed for solid materials, such as dipole coupling, chemical shift anisotropy and magnetic susceptibility differences are significantly minimized. This is attributed to the rapid rotation of the sample at the magic angle (54.7º), which renders the spectra with resolution similar to that of spectra from NMR in solution.[17]

This work focuses on the combination of solution and ^{1}H HR-MAS NMR with chemometric analysis for the chemotaxonomic classification of lichens.

Experimental

Lichens of eleven species from six genera and from two families were collected in Mato Grosso do Sul state, Brazil. ^{1}H NMR spectra were obtained either from acetonic extracts or from intact samples on a Bruker 9.4 Tesla DRX 400 spectrometer, equipped with a 5 mm multinuclear inverse detection and a 4 mm HR-MAS probehead for liquids and intact samples, respectively.

For HR-MAS measurements, the samples were spun at 5 KHz at the magic angle, and a Carr-Purcell-Meiboom-Gill (CPMG)[18,19] pulse sequence with water pre-saturation signal was used. For liquid analysis, a composite pulse sequence was used for water pre-saturation signal. All measurements were carried out in triplicate for each species, and all spectra were processed with zero filling. All ^{1}H NMR spectra were used as input variable in the *Pirouette*® software (version 2.0.2) to perform chemometric analysis using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) methods.

Results and Discussion

According to ^{1}H HR-MAS NMR spectra, most of the signals were between 3.2 and 4.0 ppm, which is a region characteristic of polyols. As for solution ^{1}H NMR, we find signals characteristic of lichen secondary metabolites, which include all the spectral region.

Chemometric analysis of ^{1}H HR-MAS NMR spectra allowed us to distinguish among families, genera and species. In Figure 1, we can identify Physciaceae (Phys.) and Parmeliaceae (Parm.) families. Only one sample presented an unusual behavior.[20]

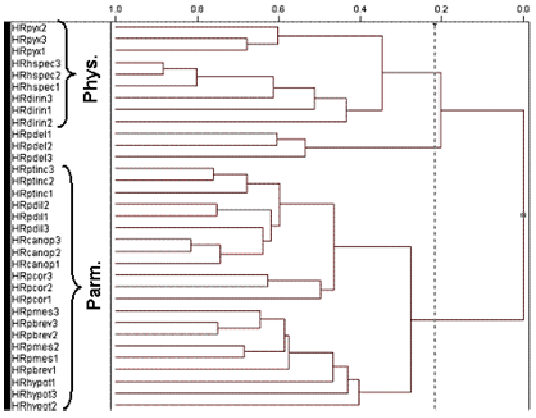

Figure 1. HCA plot of all lichen samples in HR-MAS NMR analysis (similarity 0.217)

Genera and species can also be observed. In Figure 2, chemometric analysis of lichens in acetonic extracts allowed us to distinguish between six species of the same genera (hatched area) and two others of different genera (outside of hatched area).

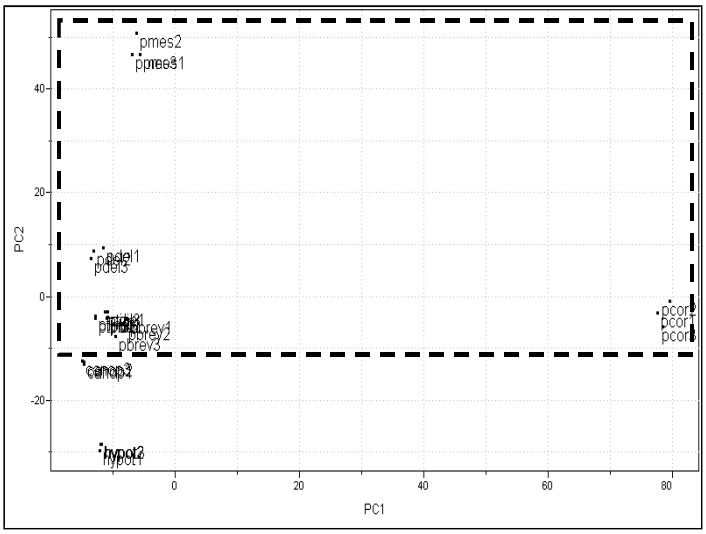

Figure 2. Scores plot of the sample of Parmeliaceae lichens using NMR in solution (PC1 xPC2, 24.1 and 11.8%, respectively)

Conclusion

Chemometric analysis of ^{1}H NMR spectra of both in solution and intact samples shows to be effective to identify different lichens and discriminate families, genera and species. Both solution and HR-MAS NMR allowed us to distinguish between genera and species. However, HR-MAS NMR showed to be more effective for classifying lichens, as families could also be identified.

Overall, in comparison with other traditional techniques, the combination of NMR spectroscopy with chemometric analysis showed to be a fast and economic method for the chemotaxonomic classification of lichens, and it could be useful to classify unknown samples.

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References

1. Kappen, L. *Report DFG*, p. 25-28, 1988;
2. Lindsay, D.C. *Bryol.*, **1978**, 81 (2): 268-276.
3. Quilhot, W.; Lighton, G.; Flores, E.; Fernandes, E.; Pena, W.; Guzman, G. *Acta Farm. Boranense*, **1987**, 6(1): 15.
4. Honda, N.K.; Vilegas, W. *Quím. Nova*, **1998**, 21(6): 110-125.
5. Carbonero, E.R.; Montai, A.V.; Stuelp, P.M.; Woranovicz-Barreira, S.M.; Gorin, P.A.J.; Iacomini, M., *Phytochem.*, **2002**, 61: 681-686.
6. Carbonero, E.R.; Montai, A.V; Mellinger, C.G.; Eliasaro, S.; Sassaki, G.L.; Gorin, P.A.J.; Iacomini, M. *Phytochem.*, **2005**, 66: 929-934,.
7. Takahashi, H.; Kon, T.; Yokota, I.; Shibata, S. *Carboh. Res.*, **1981**, 89 (1): 166-173.

8. Teixeira, A.Z.A.; Iacomini, M.; Gorin, P.A.J. *Carboh. Res.*, **1995**, 266 (2): 309.
9. Yokota, I.; Shibata, S.; Saitô, H. *Carboh. Res.*, **1979**, 69: 252-258.
10. Ward, J.L.; Harris, C.; Lewis, J.; Beale, M.H. *Phytochem.* **2003**, 62, 949.
11. Cheng, L. L.; Chang, I.; Smith, B. L.; Gonzalez, R. G. *J. Mag. Reson.*, **1998**, 135: 194-202.
12. Broberg, A.; Kenne, L., *Anal. Biochem.*, **2000**, 284: 367-374,.
13. Brescia, M.A.; Di Martino, G.; Fares, C.; Di Fonzo, N.; Platani, C., Chelli, S.; Reniero, F.; Sacco, A. *Cereal Chem.*, **2002**, 79 (2): 238.
14. Brescia, M.A.; Caldarola, V.; De Giglio, A.; Benedetti, D.; Fanizzi, F. P.; Sacco, A., *Anal. Chim. Acta*, **2002**, 458(1): 177-186.
15. Gil, A.G.; Duarte, I.F.; Delgadillo, I.; Colquhoun, I.J.; Casuscelli, F.; Humpfer, E.; Spraul, M. *J. Agric. Food Chem.*, **2000**, 48: 1524-1536.
16. Sacco, A.; Bolsi, I. N.; Massini, R.; Spraul, M.; Humpfer, E.; Cheli, S.; *J. Agric. and Food Chem.*, **1998**, 46: 4242,.
17. Claridge, T.D.W. *High-Resolution NMR Techniques in Organic Chemistry*, Tetrahedron Organic Chemistry Series, Vol. 19, Oxford, Pergamon, 1999.
18. Carr, H.R.; Purcell, E.M. *Phys. Rev.*, **1954**, 94(3): 630-638.
19. Meiboom, S.; Gill, D. *Rev. Sci. Instrum.*, **1958**, 29(8): 688-691.
20. Alcantara, G.B; Honda, N.K.; Ferreira, M.M.C.; Ferreira, A.G. Anal. Chim. Acta, **2007**, 595: 3-8.