Chemometric investigations of the multidrug resistance in strains of the phytopathogenic fungus *Penicillium digitatum*

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Key Words: *chemometrics, multidrug resistance, green mold*

Introduction

The emergence of demethylation inhibitor (DMI) resistance by pathogenic fungi represents a serious problem in agriculture and medicine (immuno-compromised patients). Fungi possess numerous resistance mechanisms, among which are CYP51 (cytochrome 51: ergosterol biosynthesis enzyme) mechanism and drug efflux pump activation. *P. digitatum* is a phytopathogenic fungus (the green mold) that causes one of the most important postharvest diseases of citrus fruits. Recent studies [1-5] have reported in detail its CYP51 mechanism, and the PMR1 pump mechanism. Therefore, it is interesting to investigate the structural patterns of toxicants to which *P. digitatum* is resistant, the mechanisms and their relations with the fungal genome. This work studies *P. digitatum* strains (DMI-resistant, moderately resistant, and sensitive), and four DMIs and three non-DMIs by means of chemometric methodologies Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and Partial Least Squares (PLS) regression. Novel types of relationships between molecular structure and fungal resistance, and between the resistance and fungal genome, were established.

Results and Discussion

Biological activities. The activity data sets for *P. digitatum* strains with respect to DMIs (triflumizole, fenarimol, bitertanol, pyrifenox) and non-DMIs (cycloheximide, 4-nitroquinoline-*N*-oxide, acriflavine) were generated from experimental EC_{50} (effective inhibitory concentration - 50% radial growth inhibition), MIC (minimal inhibitory concentration - 100% radial growth inhibition) and radial growth photographs [1-5]. 1) Data set A1: matrix (7×7) with pEC_{50} = -log(EC_{50}/mol dm^{-3}) for 7 strains and 7 toxicants; 2) Data set A2: matrix (7×6) with $pECr_{50}$ = pEC_{50}(standard strain) – pEC_{50} for 6 strains and 7 toxicants; 3) Data set A3: matrix (7×5) for 7 toxicants and 5 descriptors (*a*, *b*, *c*, |a|, |c|) from regression equations pMIC = *a* + *b* pEC_{50} and *c* = *a* / *b* for each toxicant; 4) Data set B1: matrix (39×8) describing the growth of 39 strains without toxicant, using 8 morphological descriptors (radii, circumferences and areas of fungal cultures); 5) Data set B: (39×16) obtained by extension of B1 with analogous descriptors for the same strains with triflumizole; 6) Data set C1: pEC_{50} data (92×1) from multiple measurements for 24 strains and 4 DMIs; 7) Data set C2: pEC_{50} data (29×1) for several strains and 3 non-DMIs; 8) Data set C: C1+C2. The corresponding matrices (92×6), (29×6) and (131×6) were constructed from literature data [1-5]. These are genome structure descriptors related to constitutive and toxicant-induced expression levels of *CYP51* and *PMR1* genes in diverse *P. digitatum* strains.

Activity-structure relationships (ASRs). Exploratory analyses were performed for the data sets A1-B1. The obtained results are similar, and in some cases mutually complementary. Relationships between toxicant molecular structure and strain characteristics (baseline resistance, morphology, origin/target) are visible and can be rationalized. Low DMI resistance may be described by DMIs structural patterns, what can be useful in detecting high resistant strains and discovering new antifungals.

Quantitative genome-activity relationships (QGARs). Satisfactory PLS model was obtained using the data set C1 (DMIs only, without 6 outliers): 3 principal components with 99.5% total variance, R = 0.90, Q = 0.89, SEV = 0.34, SEP = 0.33. PLS models for C2 and C were not so good due to small activity variation for non-DMIs. The exploratory analysis for C1 has shown the primary role of *CYP51* gene structure for DMI resistance, and the secondary of *PMR1* gene structure and its interaction with toxicant triflumizole.

Conclusions

Several novel activity-structure and quantitative genome-activity relationships have shown that the *P. digitatum* resistance to DMIs depends on genome structure and its interaction with toxicant, DMI molecular structure and other strain characteristics. This may aid in detecting resistance strains and development of novel antifungals.

Acknowledgements

The authors thank to FAPESP.

[1] Nakaune, R. *et al.*, *Microbiol.* **1998**, *64*, 3983.
[2] Hamamoto, H., *et al.*, *Appl. Environ. Microbiol.* **2000**, *66*, 3421.
[3] Hamamoto, H. *et al.*, *Pestic. Biochem. Physiol.* **2001**, *70*, 19.
[4] Hamamoto, H. *et al.*, *Pest. Manag. Sci.* **2001**, *57*, 839.

[5] Nakaune, R. *et al.*, *Mol. Genet. Genom*. **2002**, *267*, 179.