Comparison of genotypic and phenotypic techniques for assessing the variability of the fungus *Epicoccum nigrum*


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Aims: The diversity within a collection of worldwide isolates of *Epicoccum nigrum* has been studied using several phenotypic approaches. In addition, the abilities of phenotypic and genotypic techniques for the differentiation of a set of isolates are compared.

Methods and Results: The methodology used include the study of isozymes (acetyl esterase and alkaline phosphatase), HPLC profile of metabolites and antibiotic activities against a panel of bacteria, yeasts and filamentous fungi, and cytotoxicity against three mammalian cell lines. Two procedures for assessing the relationships within a collection of isolates, using a combination of the techniques, were evaluated, comparing the advantages and disadvantages of each method.

Conclusions: The results showed that each individual technique allows differentiation of the isolates studied to some degree and that the information provided by each technique could be considered as complementary. Genotypic techniques were more powerful than the phenotypic ones to discriminate among the strains.

Significance and Impact of the Study: This work evaluates the predictive value of several phenotypic techniques on a collection of fungal isolates, and compares the results obtained with genotypic techniques performed on the same strains.

INTRODUCTION

Discrimination and classification is one of the main goals in any culture selection process. The strategies traditionally followed to classify/de-replicate isolates include both genotypic and phenotypic methods. Historically, the most widely used were phenotypic methods such as morphological analysis, the study of biochemical or physiological features (Logan and Khan 1969), chemotaxonomy (Frisvad 1989) and isozyme analysis (Monte et al. 1990). During the last decade, methods based on the analyses of DNA polymorphisms were added to this list of methodologies: Random Amplified Polymorphic DNA (RAPD) (Williams et al. 1990), Arbitrarily Primed PCR (AP-PCR) (Welsh and McClelland 1990), microsatellite-primed PCR (Longato and Bonfante 1997), tDNA-PCR (Welsh and McClelland 1991), Amplified Ribosomal DNA Restriction Analysis (ARDRA) (Ward and Akrofi 1994) and Amplified Fragment Length Polymorphism (AFLP) (Vos et al. 1995).

Numerous reports describe the analysis of sets of microbial cultures using one or several of these procedures. However, very few have compared techniques simultaneously on the same set of isolates (Talbot et al. 1996). Variability within isolates of the fungus *Epicoccum nigrum* were evaluated in a previous paper (Arenal et al. 1999) using different DNA fingerprinting methods. *Epicoccum nigrum* is a cosmopolitan airborne and soilborne fungus and has been found to be both genotypically (Arenal et al. 1999) and phenotypically (Kilpatrick and Chilvers 1981) very variable. *Epicoccum nigrum* also produces secondary metabolites of potential biotechnological interest, including carotenoid pigments (Gribanovski-Sassu and Foppen 1967), antifungal compounds, such as flavipin (Bamford et al. 1961), epirodins (Ikawa et al. 1978), epicorazins

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