Alt a 1 Protein Family in Phylogenetic-Related *Alternaria*

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Alternaria is a genus of worldwide fungi found in different habitats such as soil, the atmosphere, plants or indoor environments. *Alternaria* species are saprobic—largely involved in the decomposition of organic material—but they can also act as animal pathogens, causing disease in humans and animals, developing infections, toxicosis and allergic diseases. *A. alternata* is considered one of the most important sources of fungal allergens worldwide and it is associated with severe asthma and respiratory status. In fact, Alt a 1, the main allergen of *A. alternata,* is an important marker for assessing the risk factor and severity of allergic respiratory disease. Another role of Alt a 1, from a evolutionary point of view, would be to define a family of proteins that would allow establishing taxonomic relationships between different fungal divisions. Finally, Alt a 1 has been shown to be a very useful marker for the identification of pathogenic molds contaminating plants and fruits.

Keywords: Alt a 1 ; fungal allergy ; asthma ; Alternaria crop contamination

1. Introduction

Alternaria is a worldwide genus of Deuteromycetes fungi found in different habitats, such as soil, the atmosphere, plants or indoor environments, that include many saprophytic and pathogenic species. The taxonomy of these fungi has mainly been based on morphological conidial characters and to a lesser extent on host association, biochemistry and metabolites [1]. Recent phylogenetic studies have made significant changes to the systematic taxonomy within *Alternaria* by elevating 26 clades to the subgeneric taxonomic status of section [1]. According to phylogenetic and morphological studies, *Alternaria* contains most *Alternaria* species with conidia succession, including important plant, human and postharvest pathogens. In spite of the fact that the majority of *Alternaria* species are saprobic—largely involved in the decomposition of organic material—many of their species are endophytic, living in various parts of crops including leaves, seeds and fruits [2]. To a lesser degree, they can act as animal pathogens, causing disease in human and animals, developing infections, toxicosis and allergic diseases [1][3]. In the field of human ailments, allergic disease is undoubtedly the most common human pathology caused by *Alternaria* [2][4][5][6]. The Global Asthma and Allergy European Network (GA2LEN) initiative funded by the European Union under the 6th Framework Programme with the purpose of addressing the growing public health concern of allergic diseases, performed a study in 14 countries of the European Community (*n* = 3034) and showed a prevalence of sensitization to *A. alternata* of 9%, ranging from 2% in Finland to 23.8% in Greece [7]. In the United States, the prevalence of *A. alternata* sensitization in the general population (aged 6 to 74 years) was about 13% [8]. In Spain, this prevalence was estimated to be around 20% [9].

2. Alt a 1 Protein Family in Phylogenetic-Related *Alternaria*

Although Alt a 1 has been a defined allergenic protein since 1991 [10][11], in 2012 it was defined and described as a unique β-barrel protein dimer found exclusively in fungi. The only allergens with a similar structure to Alt a 1 are lipocalins, which have an α-helix in addition to a β-barrel. Despite the similarities between lipocalins and Alt a 1, they are the homologs of Alt a 1 which define a distinct structural family of proteins [12].

Current phylogenetic studies have made significant changes to the systematic taxonomy of *Alternaria* by elevating 26 clades to the subgeneric taxonomic status of section [1]. *Alternaria* section consists of only 11 phylogenetic species and 1 species complex [13][14][15]. Alt a 1 gene sequences contain more parsimony-informative sites than other phylogenetic markers. Analyses of Alt a 1 gene strongly support the clustering of *Alternaria* spp. and related taxa into several species-groups: infectoria, alternata, porri, brassicicola, sonchi, radicina and embellisia group. The monophyly of the *Nimbya* group was moderately supported and the monophyly of the *Ulocladium* group was weakly supported [16]. Despite the high levels of variation in amino acid sequences, the results of in silico prediction of protein secondary structure for Alt a 1

demonstrated a high degree of structural similarity between most of the species, suggesting conservation of function [1][3][16].

According to this concept, several scholars have used Alt a 1 DNA sequence or Alt a 1 protein to identify and quantify *A. alternata* from the environment, this being of particular interest for associating *Alternaria* with sensitization or respiratory allergy [17][18][19]. Gabriel et al. were able to identify Alt a 1 homologs from *A. alternata*, *A. tenuissima*, *A. infectoria*, *U. botrytis* and *S. botryosum* using different Alt a 1 expression gene sequences. The specific sequence of Alt a 1 was able to detect an amplicon of approximately 390 bp from Alt a 1, encoding genes from species closely related taxonomically to *A. alternata*, such as *A. tenuissima*. By contrast, the PCR system using a conserved sequence of Alt a 1 homologs was able to detect an amplicon of approximately 180 bp from Alt a 1 and Alt a 1-like encoding genes from *A. alternata*, *A. tenuissima*, *A. infectoria*, *U. botrytis* and *S. botryosum* [20]. Similar results were reported by Teifoori et al., who were able to amplify Alt a 1 gene sequences from in *A. alternata* [21] and other related taxa [22][23]. All these scholars agree that the Alt a 1 sequence is an excellent tool to define a monophyletic *Alternaria-Nimbya-Embellisia-Ulocladium* and the other clade with *Stemphylium* belonging to a very close clade to the mentioned above. *A. alternata* and *A. tenuissima* are taxonomically closely related species and are placed in a different group from those including *A. infectoria, U. botrytis, and S. botryosum.* Other analyzed *Pleosporaceae*, as *Curvularia lunata* or *Drechslera tritici-repentis* did not revealed homologous genes to Alt a 1 [20]. Despite the differences in Alt a 1 gene sequences among the different *Pleosporaceae* species studied, the recognition of Alt a 1 homologs by antibodies is always relevant and, Alt a 1 can be considered an excellent tool to be used in the serological diagnosis of allergic diseases caused by *Alternaria.*

If accept the gene encoding Alt a 1-homologous-proteins is a taxonomic marker [24] and that Alt a 1, expressed in some *Pleosporaceae* species, defines a protein family [16], it would be possible that Alt a 1 would have an important biological role in the evolutionary adaptation of the *Pleosporaceae* family because Alt a 1 defines a group of phylogenetically related species. Unfortunately, so far, no data on the biological role of Alt a 1 has been conclusively elucidated.

From an allergological point of view, it could be suggested that the Alt a 1 protein, both in its native and recombinant form, is an excellent diagnostic marker to replace the *A. alternata* extract, whether used in skin tests or for the detection and quantification of specific IgE/IgG [25]. The Alt a 1 allergen, fundamentally in its native form, is currently the most accurate and effective tool for the immunotherapeutic treatment of *Alternaria* respiratory allergy [26].

Alternaria species is a common saprophyte found on many plants and other substrates worldwide. It is an opportunistic pathogen that infects many agricultural crops in the field and during postharvest storage of vegetables and fruits. *Alternaria* species have been isolated from a wide range of fruits and vegetables. Certainly, this group includes the main pathogenic fungi in agriculture and food industry, resulting in severe agricultural and economic losses [27]. Accurate identification of plant pathogens is essential for understanding epidemiology and the identification of new tools for better management of the plant pathologies and postharvest contamination [28][29].

The development of DNA technology has provided effective methods for the study of different fungi, and several genetic markers have been provided for their identification. These markers are widely used in PCR or chip detection [30]. In recent years, multigene phylogeny has been widely employed for the identification and characterization of *Alternaria* species. Molecular approaches based on barcoding the gene region or gene fragments, such as the internal transcribed spacer (ITS) [31][32][33][34], mitochondrial small subunit (mtSSU), large subunit ribosomal DNA (LSU) [33], *A. alternata* major allergen (Alt a 1) [33][35][36], glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [32][33][34][35], anonymous genomics regions (OPA 1–3 and OPA 2–1) [31][34][35], translation elongation factor 1 (TEF1) [33][34][35], RNA polymerase, the second largest subunit (RPB2) [33][34], plasma membrane, ATPase, calmodulin [33][35] and actin [35], have been used to define the monophyly of *Alternaria-Nimbya-Embellisia-Ulocladium* in the Ascomycete family *Pleosporaceae* relationships [16]. Current advances, especially in multi-gene phylogeny and comparative genomics, have made it possible to redefine and delineate the different *Alternaria* sections, with accurate molecular differentiation and identification of isolates showing that the *Alternaria* section consists of 11 phylogenetic species and 1 species complex [13][14][15].

Recently, some fungal allergens (Alt a 1 and Asp n 3) have been presented as valuable molecular markers of taxonomy and pathology/contamination in vegetables and fruits and also as molecular markers of allergenic contamination in indoor environments [24][30][37]. Gabriel et al., using primers defining the Alt a 1 encoding gen (390 bp) and the conserved region of Alt a 1 encoding gen (180 bp) from *A. alternata*, were able to detect the infection of citrus fruits by *A. alternata* at the onset of infection [30]. The conserved region was able to detect Alt a 1 homologs in several species of *Alternaria* section, but the fragment encoding of the Alt a 1 gen (390 bp) only detected *A. alternata* and *A. tenuissima*. They also suggest that this protein could play a role in the pathogenicity and virulence of *Alternaria* species [20][37]. Garrido-Arandia et al. propose

that Alt a 1 would block some plant defense mechanisms, acting as a pathogenicity factor facilitating infection by the *Alternaria* species of the plant [38].

3. Conclusions

Alt a 1 defines the respiratory allergy caused by *Alternaria*, a phylogenetic related species belonging to *Pleosporaceae* family. Although the *Alternaria* taxonomy has benefited from recent phylogenetic revisions, the basis of differentiation among the major phylogenetic clades of the group is not yet understood [1][3][13][16].

Alt a 1 is a protein associated with pathological phenomena in both animals and plants. Alt a 1 is useful for detecting and identifying indoor and outdoor amounts of the most important allergenic fungi causing respiratory allergy and sensitization. It is tacitly accepted that Alt a 1 is an important marker for assessing the risk factor and severity of allergic respiratory disease [17]. While many efforts have been made to discover the biological role of Alt a 1, no conclusive results have been reached [12][16][38][39].

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