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18S rRNA gene sequences and supraordinal classification of the Erysiphales

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Abstract: The Erysiphales (powdery mildews) exhibit conflicting morphologies and have been classified as either Pyrenomycetes or Plectomycetes. The nuclear small subunit ribosomal RNA gene was sequenced for two isolates of *Blumeria graminis* forma speciales *hordei* in order to help resolve this classification controversy. A single most parsimonious tree was obtained grouping the Erysiphales with neither the Plectomycetes nor the Pyrenomycetes, but rather placing the powdery mildews in amongst a basal assemblage of apothecial and pseudothecial fungi. Maximum likelihood tests were conducted on constraint trees exhibiting three possible morphological classifications. The results rejected the placement of the Erysiphales amongst both the Plectomycetes and Pyrenomycetes but could not reject placement amongst the Discomycetes; therefore, the powdery mildews are not Pyrenomycetes nor Plectomycetes. However, higher order classification of the Erysiphales remains unresolved, since there is no strong bootstrap support for the Erysiphales amongst the basal assemblage of apothecial and pseudothecial fungi.

Key Words: *Blumeria graminis* f.sp. *hordei,* Erysiphales, molecular evolution, powdery mildews, 18S rDNA

Classification of the Erysiphales above the ordinal level has long been controversial because the Erysiphales exhibit conflicting morphological characters. The formation of a closed ascocarp, or cleistothecium, is used to classify the Erysiphales with the Plectomycetes (Ainsworth et al., 1971; Webster, 1980). Conversely, the production of asci from a basal hymenium and the forcible discharge of their ascospores are used to classify the Erysiphales with the Pyrenomycetes (Yarwood, 1973, 1978; Alexopoulos and Mims, 1979). Based on nuclear ribosomal DNA sequence evidence, we show that the Erysiphales do not group with fungi in either the Plectomycetes or Pyrenomycetes but rather occupy a more basal position in amongst the filamentous ascomycetes along with the apothecial and pseudothecial fungi.

We have sequenced over 1700 base pairs of the nuclear encoded small-subunit (18S) rRNA gene from two isolates of *Blumeria (Erysiphe) graminis* (DC.) Speer f.sp. *hordei* (Sui259, Sui261). Miniprepped DNA of these two isolates were generously provided by Joe McDermott of the Swiss Federal Institute of Technology in Zurich. The polymerase chain reaction (PCR) was used to amplify 18S rDNA delimited by primers NS1 and NS8. PCR parameters consisted of 35 cycles with the following features: 2 min at 97 C, 1 min at 48 C, and 45 sec at 72 C, with a 4 sec per cycle extension at 72 C (Lee and Taylor, 1990; White et al., 1990). Asymmetric amplification of double stranded PCR product generated single strand template (White et al., 1990). Both strands of the DNA were sequenced by using primers NS1, NS2, NS3, NS6, NS7, NS8 (White et al., 1990), NS19-NS23 (Gargas and Taylor, 1992), and MB2, the complement of NS23.

The 18S rDNA sequence of *Blumeria graminis* f.sp. *hordei* was compared to 18 ascomycetes (alignment available upon request). The names and available GenBank accession codes are as follows: *Schizosaccharomyces pombe* Linder (X54866), *Saccharomyces cerevisiae* Meyen *ex* Hansen (J01353), *Kluyveromyces lactis* van der Walt (X51830), *Neurospora crassa* Shear & Dodge (X04971), *Chaetomium elatum* Kunze (M83257), *Microascus cirrosus* Zukal (M89993), *Ophiostoma ulmi* (Buism.) Nannf. (M83261), *Eurotium rubrum* König et al., *Talaromyces flavus* var. *macrospora* C. Benj. (M83262), *Thermoascus crustaceus* Miehe (M83263), *Eremascus albus* Eidam (M83258), *Pleospora rudis* Rabenh. *ex* Ces. & de Not., *Peziza badia* Pers., *Calicium tricolor* F. Wilson, *Ascobolus lineolatus* Brumm., *Sclerotinia sclerotiorum* (Libert) de Bary, *Leotia lubrica* Pers. *ex* Fr., *Porpidia crustulata* (Ach.) Hertel & Knoph, and *Blumeria graminis* f.sp. *hordei* (L26253).

The initial sequence alignment was produced with the Genalign option in the Intelligenetics, Inc., package. This alignment was then visually adjusted with a word processing program with color coded nucleotides. Gaps were introduced to the alignment to account for nucleotide insertions of the more divergent

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